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DEPARTMENT OF HEALTH & HUMAN SERVICES

National Institutes of Health
National Institute of
Environmental Health Sciences
P.O. Box 12233
Research Triangle Park, N.C. 27709

Memorandum

Date June 7, 1990

From Director, NIEHS

Subject Manganese in Gasoline; Toxicity Problems

To Deputy Assistant Administrator for
Pesticides and Toxic Substances

We have recently been informed about the proposed addition of manganese compounds to gasoline. This is of concern to us for the reasons we have documented in all the attachments to this memo. Manganese is a nutritional requirement as well as a toxic metal. The critical studies needed to decide whether what manganese is added to gasoline may adversely affect human health are not now available in our opinion. A digest of the materials on this issue has been assembled by my staff. In summary:

1. Mn toxicity has several similarities to lead toxicity--e.g., pregnant women, iron-deficient persons; the fetus and young child are more susceptible. Metal speciation may also be critical here. As with lead, there are several forms of Mn involved (both organic and inorganic), and they differ much in toxicity. The critical studies have not been made (also as with lead).

2. MMT can be absorbed through skin and probably readily via the nose (straight shot to the brain) and lung. These routes would give better access to CNS than orally. Most of the toxicity data (we have seen) and cited by Ethyl Corp., et al. are by oral routes and do not apply to other routes. Oral absorption of Mn can be varied (e.g, by iron deficiency) and is not well understood (what about effects of variation in gastric acidity?).

3. The CNS effects of Mn are not reversible easily, if at all, and there is no treatment for Mn poisoning effects on the CNS.

4. Presently available studies of the "fate" of Mn in gasoline do not account for the vast majority of that Mn added into the gasoline (some 1200 ugms were added and yet only 7 are accounted for?!--per mile). Where is all this Mn after burning? For example, could it have escaped in exhaust as small particles not caught by the methods used by Ethyl Corp in their studies?

5. Humans can be exposed to several oxides/salts of Mn if it (MMT) is added to gasoline. For example, Mn_3O_4 is much more toxic than MnO_2. The toxicity of MMT is in the same range as that for tetraethyl lead.

6. The epidemiology studies on Mn are primarily on humans exposed to large amounts of Mn bearing dust/fumes (as in miners, metals workers, grinders). The reported effects are often dramatic, but low dose exposures to the Mn forms in gasoline/exhaust have never been made. It is impossible to guess how applicable the presently available human studies are to the exposures we will get from gasoline.

7. Manganese may act via the same mechanisms as other heavy metals (especially lead). Synergism is possible and has not been studied for any organ system. Dr. Routt Reigart (University of South Carolina Medical School) raised the issue especially with respect to the CNS.

Please let me know if we may be of further assistance.

David P. Rall, M.D., Ph.D.

Attachments

Appendix I

A-90-16 DRAFT REPORT
IV-H-1

SUPPORT FOR CHEMICAL NOMINATION AND SELECTION
PROCESS OF THE NATIONAL TOXICOLOGY PROGRAM

NIEHS CONTRACT No. NO1-ES-5-5097

EXECUTIVE SUMMARY OF DATA

METHYLCYCLOPENTADIENYL MANGANESE TRICARBONYL

October 31, 1986

Submitted to:

National Toxicology Program
National Institutes of Health
Building 31, Room 2B-55
Bethesda, Maryland 20205

Submitted by:

Dynamac Corporation
The Dynamac Building
11140 Rockville Pike
Rockville, Maryland 20852

Rev. 1/14/87

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LIST OF ENCLOSURES

NTP EXECUTIVE SUMMARY OF DATA DRAFT

METHYLCYCLOPENTADIENYL MANGANESE TRICARBONYL*

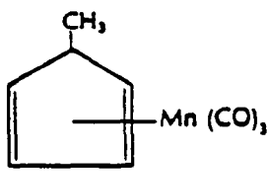
I. Chemical and Physical Information

A. Synonyms: Manganese, tricarbonyl methylcyclopentadienyl
Manganese, tricarbonyl [(1,2,3,4,5-eta)-1-methyl-2,4-cyclopentadien-1-yl]-
2-Methylcyclopentadienyl manganese tricarbonyl
MMT

B. CAS No: 12108-13-3

C. Molecular Formula: $C_9H_7MnO_3$

D. Structural Formula:



E. Molecular Weight: 218.10

F. Physical Properties:

1. Physical State: Dark orange liquid (Verschueren, 1983)
2. Melting Point: 1.5°C (Kirk-Othmer, 1981); 2.22°C (ACGIH, 1980)
3. Boiling Point: 232.8°C (Verschueren, 1983)
4. Flash Point: 110°C, closed cup (ACGIH, 1980)
5. Vapor Pressure: 0.047 mm Hg at 20°C (Verschueren, 1983)
6. Specific Gravity: 1.39 at 20°C (ACGIH, 1980)
7. Refractive Index: No information was found.
8. Solubility in Water: 70 ppm at 25°C (Verschueren, 1983)
9. Solubility in Organic Solvents: Completely soluble in hydrocarbons (Verschueren, 1983)
10. Log Octanol/Water Partition Coefficient: 3.5 estimated (USEPA, 1983)
11. Other: Faintly pleasant odor, decomposes when exposed to light (ACGIH, 1980); half-life of a few seconds in air (Verschueren, 1983)

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*The Environmental Protection Agency has nominated methycyclopentadienyl manganese tricarbonyl for three generation reproductive toxicity studies.

October 31, 1986.

II. Production/Use/Exposure/Environmental/Regulatory Data

A. Production

1. Manufacturing Process

Methylcyclopentadienyl manganese tricarbonyl (MMT) is manufactured by the gradual addition of methylcyclopentadienyl dimer to an agitated mixture of molten sodium metal and diethylene glycol dimethyl ether at 185-190°C. Anhydrous flaked manganese chloride is then added, under agitation at 165°C, to the methylcyclopentadienylsodium formed in the first step, producing bis(methylcyclopentadienyl manganese). The latter product is treated with carbon monoxide at 625 or 650 psi and 193°C to form MMT, which is isolated from the reaction mixture by vacuum distillation (Kirk-Othmer, 1981).

2. Volume

Production volume data for MMT in 1977 were not reported in the public portion of the Toxic Substances Control Act (TSCA) Chemical Substance Inventory (TSCA Inventory) (USEPA, 1985).

The U.S. International Trade Commission (USITC, 1981-1985) did not provide production volume data for MMT from 1980 to 1984 because only one manufacturer reported production of the compound in each of those years (see Section II.A.3). The USITC lists production volume data on a compound only if three or more companies manufacture the compound in a given year.

Ethyl Corporation (1983) reported production of about 4 million pounds of MMT in 1983. Exports of the compound to Canada have apparently accounted for about half of the reported domestic production in recent years (see Section II.B).

CEH (1982) reported that the compound is not produced or consumed in Western Europe or Japan. These data indicate that MMT is not imported into the United States. According to SRI International, the Ethyl Corporation manufacturing plant (see Section II.A.3) has an annual production capacity of about 10 million pounds (CEH, 1982).

3. Producers and Importers

Producers (SRI International, 1985; USITC, 1981-1985; USEPA, 1985)

Ethyl Corporation, Chemical Group
Orangeburg, SC

Ethyl Corporation (1983) reported that it is the sole domestic manufacturer of MMT; the company produces the compound only at the facility listed above.

Importers

No information was found.

4. Technical Product Composition

Ethyl Corporation markets MMT as a product of greater than 97% purity, containing a minimum of 24.4% manganese (Mn) by weight (Ethyl Corporation, 1983).

B. Uses

MMT is currently used in domestic applications as an antiknock additive in leaded regular gasoline and as a combustion improver in turbine fuel (Ethyl Corporation, 1983). Ethyl sells approximately 2 million pounds of MMT annually as an antiknock additive

for use at an average concentration of 0.03 to 0.05 g Mn/U.S. gallon of leaded regular gasoline. Sales of the compound as a combustion improver total about 100,000 pounds annually, worldwide. Use of the compound in this application is decreasing due to the greater efficiency of modern turbine engines.

MMT is also currently used as an antiknock additive in essentially all of the unleaded gasoline marketed in Canada (Ethyl Corporation, 1983). The compound is added to unleaded premium gasoline at a concentration of about 0.06 g Mn/U.S. gallon and to unleaded regular at about 0.04 g Mn/U.S. gallon. A maximum concentration of 0.068 g Mn/U.S gallon is allowed under Canadian law.

Prior to a ban on the use of MMT as an additive to domestic unleaded gasoline by the EPA, effective September 1978, 60% of domestic refining capacity used MMT in unleaded gasoline in 1977 (Ethyl Corporation, 1983; CEH, 1982). The EPA banned the compound from domestic unleaded fuels based on evidence that the use of MMT results in a slight increase in hydrocarbon emissions at the tail pipe (Ethyl Corporation, 1986).

C. Occupational Exposure

The National Occupational Hazard Survey (NOHS), conducted by the National Institute for Occupational Safety and Health (NIOSH) from 1972 to 1974, estimated that 22,967 workers in 720 plants were potentially exposed to MMT in the workplace (NIOSH, 1976). These estimates were derived from observations of the actual use of MMT (less than 1% of total estimate) and the use of generic products suspected of containing the compound (greater than 99% of total estimate). The largest number of exposed workers was in the machinery (except electrical) industry (refer to Enclosure 1).

The occupational groups with the largest number of exposed workers were drill press operators, tool and die makers, lathe and milling machine operators, machinists, packers and wrappers (except meat and produce), and machine operators (not specified) (refer to Enclosure 2).

NIOSH conducted a second workplace survey, the National Occupational Exposure Survey (NOES), from 1980 to 1983 (NIOSH, 1984). Preliminary data from NOES indicated that 1,082 workers in 10 plants were potentially exposed to the compound in the workplace in 1980. All of these workers were employed in the petroleum and coal products industry; most of them were janitors and cleaners and industrial truck and tractor equipment operators (refer to Enclosure 3). Unlike NOHS, the NOES estimates were based only on observations in which the surveyor observed the actual use of the compound.

Ethyl Corporation (1986) reported that MMT is currently used only at refineries. Occupational exposure to MMT is virtually absent during blending and manufacturing processes, which are closed-system operations. The only documented exposures to MMT have resulted from accidental sprays or spills of MMT during material transfer operations. Approximately 70 workers are potentially exposed to MMT in manufacturing, handling, or maintenance at Ethyl's MMT plant. Refinery and transport workers are also potentially exposed to MMT.

The NIOSH Tradename Ingredient Data Base of NOHS listed MMT as a constituent of one product used in industrial applications (refer to Enclosure 4) (NIOSH, 1976). The concentration of MMT in the product was 96%.

The American Conference of Governmental Industrial Hygienists (ACGIH) has adopted an 8-hour time-weighted average threshold limit value of 0.2 mg Mn/m^3 and a short-term exposure limit

(STEL) threshold limit value of 0.6 mg Mn/m^3 for MMT (ACGIH, 1985). A "skin" notation was designated, indicating the potential for dermal absorption of toxic amounts of the compound. ACGIH further recommended that appropriate measures be taken to prevent dermal contact and cutaneous absorption. The ACGIH (1984) proposed that the adopted STEL for MMT be deleted on the basis that the information available relates to chronic effects. The deletion of this value is recommended until acute toxicological data and industrial hygiene experience become available to provide a basis for quantifying what the STEL should be.

Ethyl Corporation (1983) stated that the results of periodic monitoring of operators at their manufacturing plant indicate that most concentrations of MMT in workplace air have been less than 40 µg Mn/m^3, the detection limit of their personal monitoring method. Exposures as high as 100 µg Mn/m^3 have been detected in a few instances.

D. Consumer Exposure

Exposures to MMT may occur through its use as an antiknock additive in leaded regular gasoline. Low-level inhalation or dermal exposure may occur during self-service pumping of the gasoline.

E. Environmental Data

Coe et al. (1980, as cited in USEPA, 1983) were unable to detect MMT (detection limit 0.05 ng MMT/m^3) in air in Toronto, Canada, where MMT is used extensively in unleaded gasoline. The only location in which MMT was found in air was an underground parking garage where levels of 0.1 to 0.3 ng/m^3 were detected.

MMT in gasoline is photolytically unstable, decomposing in the presence of both light and oxygen (Ter Haar et al., 1975). Almost all of the manganese is converted to a mixture of solid

manganese oxides and carbonates. The organic portion of the solid decomposition products appears to be a complex mixture of acids, esters, and hydrocarbon polymers. A half-life of 8-18 seconds was calculated for MMT by Ter Haar et al. (1975). Although they were unable to experimentally determine a definite half-life, the authors concluded that the survival of MMT in the atmosphere would be either seconds or minutes at the longest. The log octanol/water partition coefficient of MMT has been estimated to be 3.5, suggesting the potential for bioconcentration.

F. Regulatory Status

The Occupational Safety and Health Administration has not established a permissible exposure limit for MMT (OSHA, 1983).

MMT has not been scored or studied by the TSCA Interagency Testing Committee (ITS, 1985).

MMT is regulated as an automobile fuel additive under Section 211f of the Clean Air Act (USEPA, 1981). This regulation prohibits the commercial use of certain automotive fuels and fuel additives. Ethyl Corporation applied for a waiver of this prohibition to permit the use of MMT at a concentration of up to 1/64 g of Mn per gallon of unleaded gasoline; however, this waiver was denied. The use of MMT in unleaded gasoline has not been approved because of concern that its use results in a slight increase in hydrocarbon emissions at the tail pipe (Ethyl Corporation, 1986).

No other Federal regulations relating to MMT were found.

III. Toxicological Effects

A. Human Data

1. Acute: No cases of overexposure resulting in lethality in

humans were found. Ethyl Corporation (1977) reported that MMT liquid produces a slight burning sensation on the skin and MMT vapors produce a metallic taste when inhaled. In unsubstantiated reports, six men with skin exposure for up to 30 minutes, showed signs and symptoms that included headache, nausea, gastrointestinal discomfort, dyspnea, chest tightness, and paresthesia. All signs and symptoms appeared 5 minutes to 1 hour after exposure and completely subsided in 2 hours in four cases. Two workers reported a vague abdominal distress that persisted for 2 days. A well-documented incident involved the case of four employees at a refinery who were exposed to MMT vapors for approximately 5 minutes while pouring 25 gallons of MMT into a large steam-heated pot. No symptoms were reported in any of the four workers. Manganese concentrations in the exposed workers' urine taken 3 hours after the incident were 23, 87, 20, and 10 µg/L. Twenty-three hours after exposure, urine samples contained 8, 22, 5, and 10 µg Mn/L, respectively. The workers were symptom free, and no adverse effects have since been reported.

In another exposure incident, the clothes of two men were wetted by MMT during the pumping of MMT out of a drum. Although their hands and face were protected by rubber gloves and air masks, the remainder of their bodies were moistened by the liquid for approximately 1.5 hours. The men complained of a slight burning sensation on their skin. In both men, hematology parameters, blood pressure, and pulse were normal and muscular coordination was good. Urine Mn levels measured on the day of exposure were 137 and 46 µg/L. A few weeks following exposure, urine Mn levels were within the normal range, and no adverse effects had developed.

Overexposure to MMT may affect the central nervous system and lead to convulsions, respiratory depression, cyanosis, and coma. Additional adverse effects following overexposure

can include labored breathing, lethargy, lacrimation, eye inflammation, and nasal discharge (Ethyl Corporation, 1976).

MMT is not irritating to the skin on single contact and is not known to cause cutaneous sensitization. However, it does appear to penetrate the skin very rapidly; 5-15 mL of MMT spilled on the hand and wrist of a worker was claimed to have caused the symptoms of "thick tongue," giddiness, nausea, and headache within 3-5 minutes (ACGIH, 1980).

2. Epidemiological Evidence/Case Reports: No information was found in the information sources searched.

3. Chemical Disposition: No information was found.

4. Biochemical Effects: No information was found.

5. Carcinogenicity/Chronic: No information was found.

6. Teratogenicity and Reproductive Effects: No information was found.

B. Animal Data

1. Acute: The acute systemic toxicity data for MMT in various laboratory animals are presented in Table 1.

Pfitzer et al. (as cited in NAS, 1973) investigated the acute toxicity of MMT in five laboratory species by the oral route and two species by the dermal route. The LD_{50} values are included in Table 1. In general, MMT toxicity was dependent on the species (with rats the most susceptible, followed by rabbits, mice, dogs, and guinea pigs). Additionally, females were more sensitive than males, and oral toxicity was considerably greater than skin toxicity. Toxic responses appeared promptly after exposure regardless of species or route and included mild excitement and hyperactivity, tremors,

severe tonic spasms, weakness, slow and labored respiration, occasional mild clonic convulsions, and terminal coma. Animals surviving convulsive episodes failed to thrive, lost weight rapidly, and died after a few days. Primary pathological effects occurred in the kidneys, liver, and lungs.

Hakkinen and Haschek (1982) also compared species differences in acute toxicity. MMT was administered by intraperitoneal injection into mice, rats, and hamsters. The LD_{50} values are presented in Table 1. The most sensitive species was the rat, followed by the mouse and hamster. Death, preceded by dyspnea, generally occurred in the first 72 hours following treatment. Salivation, eye irritation, rough hair coat, and weakness were also noted. At necropsy, lung wet weight was increased, and mottling and/or pallor of the liver were frequently observed.

The authors also studied the pulmonary toxicity of MMT in these three species by histopathological and biochemical analyses. Mice, rats, and hamsters received a single intraperitoneal injection of purified MMT in corn oil at doses of 120, 5.0, and 180.0 mg/kg, respectively. Control animals received corn oil.

Pulmonary effects, in particular, interstitial pneumonitis, Clara cell necrosis, and bronchiolar damage, were observed in all species by 1-2 days. In addition to the pulmonary changes, histopathologic changes were present in the kidneys (all species), liver (mouse and hamster), and adrenals (mouse) 1 and 2 days after MMT treatment. MMT treatment significantly ($p < 0.05$) increased <u>in vivo</u> incorporation of thymidine into lung DNA within 1-2 days in all three species. Peak incorporation occurred on day 4 for the mice and on day 2 for the hamsters and rats. These results suggest that the mouse, rat, and hamster have different susceptibilities to MMT-induced lung injury.

Table 1. Acute Toxicity of MMT in Laboratory Animals

Species	Strain	Route	No./Sex	Dose	Effects	Reference
Mouse	CD-1	Orl	48/F	28–320 mg/kg (10% w/v solution)	LD_{50}: 230 mg/kg (95% confidence limit 167–293 mg/kg)	Hinderer (1979)
			12/F	450, 635 mg/kg (20% w/v solution)	Urinary staining; pilo-erection; mottling and discoloration of the liver; intestines were fluid filled and spotted.	
Mouse	—[a]	Orl	-/-	—	Approximate LD_{50}: 350 mg/kg	Pfitzer et al. (as cited in NAS, 1973)
Mouse	—	Orl	64/-	105–593 mg/kg	LD_{50}: 251.9 mg/kg (95% confidence limit 232.6–280.5 mg/kg)	Ohnishi (1978)
Mouse	CFI	Orl	61/M	1.0–500 mg/kg	LD_{50}: 34 mg/kg	Majima (1985)
			48/F		LD_{50}: 60 mg/kg	
Mouse	—	Ihl	-/-	300–400 mg/m^3/1 hr 500–700 mg/m^3/1 hr 1000 mg/m^3/1 hr	Some mortality 50% mortality 100% mortality	Ohnishi (1978)
			64/-	32.0–111 mg/m^3/4 hr	LC_{50}: 58.6 mg/m^3	
		Ip	64/-	105–593 mg/kg	LD_{50}: 151.5 mg/kg (95% confidence limit 139.5–164.7 mg/kg)	
Mouse	BALB/C	Ip	16/F	100–174 mg/kg	LD_{50}: 138 mg/kg (95% confidence limit 120–159 mg/kg)	Hakkinen and Haschek (1982)
Rat	COBS	Orl	80/-	15–150 mg/kg	LD_{50}: 58 mg/kg Dose related histopatho-logical changes were found in the lung, liver, and kidneys of surviving animals	Hysell et al. (1974)
Rat	Sprague-Dawley	Orl	40/M-F	10% w/v solution	LD_{50}: 58 mg/kg (95% confidence limit 37.4–89.9 mg/kg) Salivation, weakness,	Hinderer (1979)

Table 1. Acute Toxicity of MMT in Laboratory Animals (continued)

Species	Strain	Route	No./Sex	Dose	Effects	Reference
					and diarrhea; lungs were dark red and the intestinal tract and viscera were discolored. Females were more sensitive than males.	
Rat	Sprague-Dawley	Orl	20/M	30.0-118.0 mg/kg	LD_{50}: 50 mg/kg (95% confidence limit 38-67 mg/kg)	Hanzlik et al. (1980b)
Rat	—	Orl	-/-	—	Approximate LD_{50}: 9.0-176 mg/kg	Pfitzer et al. (a cited in NAS, 197
Rat	Sprague-Dawley	Ihl	90/M	-/1 hr	LC_{50}: 247 mg/m^3 (95% confidence limit 229-271 mg/m^3) Decreased activity, slight conjunctivitis, dyspnea, and lung hemorrhage.	Hinderer (1979)
				-/4 hr	LC_{50}: 76 mg/m^3 (95% confidence limit 67-87 mg/m^3) Decreased activity, dyspnea, eye irritation, weight loss, and minimal occurrence of hemorrhagic foci in the lungs.	
Rat	—	Skn	-/-	10% solution in peanut oil/6 hrs	Toxic at 665 mg/kg	Pfitzer et al. (a cited in NAS, 197
Rat	Sprague-Dawley	Ipr	16/M	9.5-76 mg/kg	LD_{50}: 23 mg/kg (95% confidence limit 13-38 mg/kg)	Hanzlik et al. (1980b)
Rat	Albino	Ipr	16/F	2.5-20 mg/kg	LD_{50}: 6 mg/kg (95% confidence limit 4-8 mg/kg)	Hakkinen and Haschek (1982)
Hamster	LV_6/LAK	Ipr	16/F	120-405 mg/kg	LD_{50}: 270 mg/kg (95% confidence limit 213-341 mg/kg)	Hakkinen and Haschek (1982)
Guinea pig	—	Orl	-/-	—	Approximate LD_{50}: 900 mg/kg	Pfitzer et al. (a cited in NAS, 197

Table I. Acute Toxicity of MMT in Laboratory Animals (continued)

Species	Strain	Route	No./Sex	Dose	Effects	Reference
Rabbit	—	Orl	-/-	—	Approximate LD_{50}: 95 mg/kg	Pfitzer et al. (as cited in NAS, 1973)
Rabbit	—	Skn	-/M	Undiluted	Approximate LD_{50}: 1700 mg/kg (24 hr)	Pfitzer et al. (as cited in NAS, 1973)
Rabbit	—	Skn	-/-	Neat	LD_{50}: 140 mg/kg[b] (95% confidence limit 122-159 mg/kg) LD_{50}: 196.7 mg/kg[b] (95% confidence limit 151.9-254.7 mg/kg) LD_{50}: 420 mg/kg[b] (95% confidence limit 170-670 mg/kg) LD_{50}: 795 mg/kg[b] (95% confidence limit 568-1113 mg/kg) Toxicity varied from polyapnea to vocalization, excitation, ataxia, tremors, cyanosis, and convulsions. Body weight loss, erythema, and edema were noted. Gross pathology revealed bloody diarrhea; lung abnormalities; discoloration of the liver, kidneys, and spleen; congested kidneys; and swollen livers, kidneys, and spleens.	Hinderer (1979)
Dog	—	Orl	-/-	—	Approximate LD_{50}: >600 mg/kg	Pfitzer et al. (as cited in NAS, 1973)

[a]Data not provided.
[b]Dermal LD_{50} studies were performed at different laboratories and summarized by Hinderer (1979).

Hanzlik et al. (1980b) studied the toxicity of MMT in rats following oral and intraperitoneal administration. The LD_{50} values are presented in Table 1. Microscopic examinations of animals that died within 24 hours revealed that their lungs were grossly distended with a blood-containing fluid. Injury to the liver and kidneys was very minor when compared to the lungs.

The authors also investigated the toxicity of MMT in rats following phenobarbital (PB) pretreatment of 60 mg/kg given intraperitoneally for 3 days. A statistically significant ($p < 0.02$) difference in survival was observed, with 10/10 pretreated rats surviving compared to 1/10 nonpretreated rats following a single oral dose of 125 mg/kg MMT (2.5 times the LD_{50} dose for this route). PB pretreatment was also found to shift the site of tissue injury from the lungs to the liver. Liver injury was evidenced in pretreated animals by a significant increase ($p < 0.05$) in plasma glutamic pyruvic transaminase levels, and a significant decrease ($p < 0.05$) in the glucose-6-phosphatase content of the liver. In addition, following an intraperitoneal injection of ^{3}H-MMT (30 mg/kg), the Mn concentration in the bile of pretreated animals was approximately 50- to 300-fold higher than that of bile from non-treated rats. Following an intravenous injection of ^{3}H-MMT (10 mg/kg) to PB-pretreated and control rats, the cumulative biliary excretion of MMT metabolites was significantly greater ($p < 0.05$) for the pretreated group when compared to the control group.

Haschek et al. (1982) studied the pulmonary toxicity induced by MMT in mice and rats. Female BALB/C mice and male Fischer rats (number not specified) received a single intraperitoneal injection of MMT in corn oil at a dose of 120 and 8.4 mg/kg, respectively. Control animals received corn oil only. MMT produced selective necrosis of non-ciliated bronchiolar epithelial cells (Clara cells) in both species; however, Clara cell necrosis was more severe in the mouse than in the rat.

In another experiment, pretreatment of mice with piperonyl butoxide (1600 mg/kg, administered intraperitoneally), an inhibitor of the mixed-function oxidase system, enhanced pulmonary toxicity and mortality following a single interperitoneal injection of 90 mg/kg MMT.

Witschi et al. (1981a) studied the effect of oxygen (O_2) exposure on MMT-induced lung damage. Seventeen female BALB/C mice were given a single intraperitoneal injection of 120 mg/kg of MMT in corn oil. A control group consisting of 17 animals received corn oil only. Nine treated and nine control animals were exposed to 70% O_2 for 6 days and then returned to room air. The extent of fibrotic changes in the lungs was quantitated by measuring total lung hydroxyproline 3 weeks after the injections. Total lung collagen was significantly increased ($p < 0.05$) only in mice exposed to MMT + O_2.

In a later study, Hakkinen et al. (1983) provided evidence for a species difference in response to MMT-induced lung toxicity following exposure to O_2. Female BALB/C mice and CD/CR rats received a single intraperitoneal injection of MMT in corn oil at 120 and 5 mg/kg, respectively. Control groups received corn oil by the same route. Immediately after each treatment, half of the MMT treated animals and half of the controls were exposed for 6 days to 80% O_2 and then returned to normal room air. Animals were sacrificed 3 weeks after the initial treatment of MMT.

In the mice, MMT alone caused a nonsignificant increase in total lung hydroxyproline content. The combined treatment of MMT + O_2 produced interstitial fibrosis and a statistically significant ($p < 0.05$) increase in lung hydroxyproline when compared to both the corn oil controls or animals treated with MMT alone.

In the rats, MMT alone produced a significant increase ($p < 0.05$) in total lung hydroxyproline content. Oxygen exposure failed to further increase lung collagen content. Histologic examination revealed that lungs of rats exposed to MMT + O_2 were indistinguishable from rats treated with MMT alone.

Two reports have described irritation studies of MMT. In one, Campbell et al. (1975) scored MMT as a nonirritant following a dermal irritation study using six male albino rabbits. MMT (0.1 mL neat) was applied to both abraded and intact skin. In the other, Hinderer (1979) tested MMT for dermal and ocular irritation in groups of six rabbits (strain and sex not specified). MMT (dose not specified) was found to be a moderate skin irritant, inducing erythema and slight edema in both abraded and intact skin. The author reported that MMT did not induce eye irritation; however, no details were provided.

2. Chemical Disposition: No specific studies were found that measured the degree of absorption of MMT.

Ohnishi (1978) exposed mice (strain, sex, and number not specified) to MMT mist and vapor at concentrations of 100-1000 mg/m^3 for 1 hour or at 32-111 mg/m^3 for 4 hours. After 1 hour of exposure, organic Mn was higher in the liver and kidneys than in the lungs and brain. After the 4-hour exposure, inorganic and organic Mn was detected in a dose-dependent manner in the livers, lungs, and kidneys.

The author also exposed six mice to MMT at concentrations of 0.1-0.3 and 1-2 mg/m^3, 22 hours/day for 21 days, and three rats to 0.1-0.3, 1-2, and 5-7 mg/m^3, 22 hours/day for 4

days. In the mice, Mn content was dose-dependent in the kidneys, liver, lungs, spleen, and blood. In the rats exposed for 4 days, Mn was measured in all the organs, but the concentrations varied.

Majima (1985) measured the Mn concentration in the livers and kidneys of male and female CF-1 and C57BL/6 mice (number not specified) following a single oral dose of 10 mg/kg MMT. The highest Mn concentrations were measured in the livers and kidneys of male CF-1 mice on days 1 and 2. Mn concentrations had returned to control values in the males and females of both species 3-4 days postdosing.

Hysell et al. (1974) determined the concentrations of Mn in rat tissues following a single oral dose of MMT. Seventy COBS rats received MMT in Wesson oil at doses ranging from 15 to 150 mg/kg. A control group of 10 rats received only Wesson oil. The Mn concentration in tissues from animals dying after exposure to MMT was dose dependent. The highest concentrations were found in the duodenum, followed by the kidney, liver, lung, brain, and heart. By 14 days post-exposure, the Mn concentration in most tissues of exposed animals were similar to the controls. However, Mn content in the lung was approximately 6- to 12-fold higher in the exposed animals.

Hanzlik et al. (1979) conducted a series of <u>in vitro</u> experiments designed to determine if the cytochrome P-450 system was important in the biotransformation of MMT. Rat liver microsomes were prepared from PB-pretreated (60 mg/kg given intraperitoneally for 3 days) rats and control (non-pretreated) rats and incubated with 0.5 mM MMT under various conditions. The rate of MMT metabolism in the presence of PB-induced microsomes was approximately twice

that of the controls. MMT metabolism was shown to require molecular oxygen and the NADPH-generating system, and it was inhibited by both carbon monoxide and the cytochrome P-450 inhibitor N-decylimidazole.

Hanzlik et al. (1980a) studied the metabolites of MMT formed in rats pretreated with phenobarbital (PB). They found that oral administration of tritiated ^{3}(H) MMT (125 mg/kg) to rats that had been given daily doses of sodium phenobarbital (60 mg/kg) for 3 days resulted in excretion of 81% of the total radioactivity in urine, and 2 to 4% in feces within 48 hours. The two major metabolites identified in the urine, $(CO)_3MnC_5H_4COOH$ and $(CO)_3MnC_5H_4CH_2OH$, amounted to 67% and 14% of the total urinary tritium, respectively. These metabolites were excreted in substantial quantities in bile but underwent reabsorption and excretion by the kidney. The authors also studied the biliary excretion of MMT metabolites following intravenous administration of ^{3}H-MMT (10 mg/kg) in control and PB pretreated rats. The results indicated that PB pretreatment doubled the rate of biliary excretion of MMT metabolites. In another experiment the authors studied <u>in vitro</u> kinetics of MMT metabolism by lung and liver microsomes prepared from control and PB treated rats. MMT was rapidly metabolized by a cytochrome P-450 dependent process inducible in liver but not in lung microsomes.

Moore et al. (1974) studied whole-body retention, excretion, and tissue distribution of ^{54}Mn following oral and intravenous dosing of ^{54}Mn-labeled MMT in male Charles River rats. An initial rapid excretion of most of the ^{54}Mn occurred following both routes of exposure. Analysis of the urine and feces after dosing indicated that MMT was rapidly metabolized, and that the ^{54}Mn was excreted in the inorganic form. The liver, kidneys, and lungs contained the highest concentrations of ^{54}Mn. High levels of ^{54}Mn were also found in the urine. Results of <u>in vitro</u> biotrans-

formation assays revealed that the liver showed the highest activity for MMT metabolism. MMT was also metabolized in the lungs, kidneys, and, to a small extent, in the brain.

3. Biochemical Effects: Autissier et al. (1977, as cited in USEPA, 1983) studied the effects of MMT on oxidative phosphorylation in rat liver mitochondria. MMT inhibited both electron and energy transfer. The manganese tricarbonyl moiety appeared to be responsible for these effects.

Gianutsos and Murray (1982) examined the changes in the concentrations of dopamine (DA), gamma-aminobutyric acid (GABA), and choline acetyltransferase (CAT) in the brains of male CD-1 mice following long-term administration of MMT. Mice received subcutaneous injections of MMT, diluted in propylene glycol, at doses of 10, 20, or 80 mg/kg on alternate days for up to 3 weeks. Control mice received subcutaneous injections of propylene glycol on the same schedule. Twenty-four hours later the animals were sacrificed, and levels of DA, GABA, and CAT were measured in various areas of the brain. DA was significantly ($p < 0.05$) reduced in both the olfactory tubercle and striatum in the 80 mg/kg group and in the striatum in the 20 mg/kg group. Brain Mn concentrations were twofold higher in the 80 mg/kg group than in the control group (1.44 vs. 0.60 µg/g wet weight). GABA levels were significantly ($p < 0.05$) elevated at 80 mg/kg in the striatum and substantia nigra, but not in the cerebellum. The activity of CAT was unchanged in all the brain regions examined.

In order to determine if the effects of MMT on brain DA and GABA were the result of long-term administration, other groups of mice received single injections of MMT (80 mg/kg) and were sacrificed either 1 or 21 days after injection. There were no significant changes in DA or GABA concentrations in any of the brain regions examined.

4. Prechronic: Pfitzer et al. (as cited in NAS, 1973) exposed mice, rats, guinea pigs, rabbits, cats, and dogs (number, strain, and sex not specified) by inhalation to MMT for 7 hours/day, 5 days/week for up to 30 weeks. Concentrations of 14-17 mg/m^3 of MMT produced mortality in mice and rats but not in the other species. Lower concentrations produced no deaths in any species (no details were provided). Toxic responses appeared promptly after exposure, and included mild excitement and hyperactivity, tremors, severe tonic spasms, weakness, slow and labored respiration, occasional mild clonic convulsions, and terminal coma. Animals surviving convulsive episodes failed to thrive, lost weight rapidly, and died after a few days. Primary pathological changes occurred in the pulmonary system.

Ohnishi (1978) exposed groups of mice and rats (strain, sex, and number of animals not specified) to MMT vapors at concentrations of 5-7 mg/m^3 for 22 hours/day for 28 days or for 6 hours/day for 28 days. Animals in all groups showed a marked decrease in body weight and a high mortality. Rats and mice were also exposed to MMT at concentrations of 1-2 mg/m^3 for 22 hours/day for 28 days. These animals showed a suppressed increase in body weight and slight histological changes in the lung. Exposure of rats and mice to 0.1-0.3 mg/m^3 MMT for 22 hours/day for 28 days resulted in no abnormal findings. No further details were provided in the English abstract of the paper.

Ethyl Corporation (1978b) conducted a 12-week inhalation toxicity study of MMT in Swiss mice, Sprague-Dawley rats, and cynomolgus monkeys. There were three test groups/species, plus an untreated control group/species. Each group was comprised of 10 animals/sex, except the monkeys, where only 6 males/group were used. The animals were exposed to MMT vapors at concentrations of 0.0 µg/L (Group I), 0.3 µg/L (Group II), 3.0 µg/L (Group III), and 30.0

µg/L (Group IV), 6 hours/day, 5 days/week for 12 weeks. Animals were observed daily for mortality and clinical signs of toxicity. Body weights were recorded prior to exposure, twice per week for the first 2 weeks, and weekly thereafter. A standard battery of hematology, serum chemistry, and urinalysis tests were run on one-half of the rats and all of the monkeys at termination (12 weeks). Hematology tests were also conducted at 6 weeks. After 12 weeks of exposure all surviving mice, rats, and one-half (3) of the monkeys in each group were sacrificed, and gross necropsies were performed. The remaining three monkeys were sacrificed 14 days postexposure. At necropsy, the heart, liver, kidneys, spleen, brain, lungs, trachea, and gonads were weighed for organ weight/body weight analyses. A detailed histopathological examination was performed on five male and five female mice and rats from Group I (control) and Group IV (30.0 µg/L), as well as from all monkeys.

In the mouse study, high mortality (20% of the males and 50% of the females) and severe weight loss resulted in the sacrifice of Group IV animals after 5 weeks of exposure. Decreases in body weight were seen in these animals within 2 weeks, along with rough hair coat, lethargy, and dyspnea. The lungs showed varying degrees of hyperplasia, metaplasia, epithelial erosions, and fibrosis. Group III mice showed decreases in body weight later in the study period. In addition, the kidney and liver (female) and kidney (male) weights were elevated in this group. The female mice in Group II showed an increase in the liver and kidney weights and a decrease in the heart weight.

In the rat study, deaths occurred in three females in Group III and in one male and two females in Group IV. Both sexes in Group IV showed loss of body weight within 2 weeks, and exhibited rough hair coat, lethargy, and dyspnea. No hemato-

logic effects were observed at any level. Blood urea nitrogen was elevated, and glucose was depressed in both sexes in all MMT-exposed groups. Serum alkaline phosphatase was elevated in both sexes in Group IV.

Exposure-related histologic alterations were observed in the lungs of rats in the highest exposure group, including an increase in alveolor macrophages, pneumonitis, pleuritis, and alveolar wall thickening.

In the monkey study, no mortality or treatment-related effects in body weight, hematology, organ weight parameters, or calcium and phosphorus levels were seen. With the exception of vacuolation in the white matter of the brain stem and cerebellar folia, no exposure-related microscopic alterations were found in the monkeys. There was minimal vacuolation in three of six monkeys in Group III, but there was moderate vacuolation in five of six monkeys in Group IV.

Results from this study indicate that the mouse is the species most sensitive to MMT vapor exposures, followed by the rat and monkey, respectively. There also appears to be a sex-related difference in response, with female rodents being more sensitive than male rodents.

Pfitzer et al. (as cited in NAS, 1973) conducted studies following repeated dermal applications (species, strain, sex, number of animals, and number of applications not specified) of MMT added to gasoline at concentrations up to 16 mg/mL. No adverse effects were observed that were not attributable to the gasoline itself.

5. Carcinogenicity/Chronic: Witschi et al. (1981b) investigated the lung tumor-promoting potential of MMT in strain A/J mice. Sixty female mice were injected intraperitoneally with 500 mg/kg urethan, and an equal number of mice received

0.9% NaCl. A week later, 30 mice each from the urethan and NaCl treatment groups received 80 mg/kg MMT in corn oil intraperitoneally (for a total of six weekly injections), while the remaining mice from the urethan and NaCl treatment groups received corn oil alone. All animals were sacrificed 4 months following urethan treatment. MMT failed to enhance lung tumor formation in mice treated with urethan. There was 100% incidence in both the urethan + MMT and the urethan + corn oil groups. No increase in multiplicity of tumors resulted (7.6 vs. 8.3 tumors/mouse in urethan + MMT and urethan + corn oil groups, respectively). MMT alone did not increase the incidence of spontaneously occurring lung tumors (11% in 0.9% NaCl + MMT vs. 13% in 0.9% NaCl + corn oil groups).

6. Teratogenicity and Reproductive Effects: The teratogenic potential of MMT was investigated using Charles River COBS CD rats (Ethyl Corporation, 1979). Four treatment groups of 25 rats each and a control group consisting of 125 females were used. MMT was administered in corn oil at dose levels of 2.0, 4.5, 6.5, and 9.0 mg/kg/day orally by gavage on days 6 through 15 of gestation. The control group received corn oil only. Cesarean sections were performed on the surviving females on day 20 of gestation. Females were observed daily for mortality and clinical signs of toxicity. Implantation sites, the number of total implantations and corpora lutea, early and late resorptions, and maternal liver weights were recorded. All fetuses were examined for body weights, crown-rump length, sex, and external and visceral or skeletal malformations and variations.

At the dose level of 9.0 mg/kg/day, a slight increase in matting and staining of the anogenital haircoat was observed. There were no biologically meaningful differences in appearance and behavior in the other dose groups. One maternal death occurred in the 9.0 mg/kg/day group, and was attributed

to pneumonia. There were no treatment-related differences in reproductive parameters, e.g., resorptions, implantations and viable fetuses. A reduction in mean maternal body weight gain over the entire gestation period was noted in the dams from all the test groups when compared to the control group. A slight reduction in mean fetal body weight was observed in all the test groups as compared to the control group. The only malformation observed was an increased incidence of bent ribs at all the MMT dose levels when compared to the control group. Based on the historical control data, bent ribs were considered a common finding in CD rats.

Ethyl Corporation (1978a) conducted a Segment II teratology study with MMT in rats. Pregnant Long-Evans female rats received MMT in corn oil orally by gavage at dose levels of 5, 10, 20, or 40 mg/kg/day on days 6 through 15 of gestation. Control rats received corn oil. The parameters evaluated included maternal weight gain, physical observations, pregnancy, mortality, and reproduction data (implantations, number of fetuses, and resorptions). Fetal parameters included viability indices, fetal weight, size, sex ratios, and ossification variations. Teratology conclusions were based on fetal external, soft tissue, and skeletal abnormalities.

At dose levels of 5 and 10 mg/kg/day, maternal weight gain during the treatment period was significantly lower ($p < 0.01$) when compared to controls. Additionally, the dams suffered from epistaxis, rapid breathing, and urinary incontinence. Reproduction and fetal data were comparable to the control group. A slight increase (not statistically significant) in ocular malformations was observed at the 10 mg/kg dose level. No embryotoxicity was noted.

At the 20 mg/kg dose level, MMT caused high maternal mortality (70%), a significant ($p < 0.01$) reduction in maternal

body weight gain, decreased rate of pregnancy, cachexia, alopecia, and dehydration. Adrenal enlargement was also noted in several females. Only 55 fetuses (5 litters) were recovered for examination. There was a significant ($p < 0.01$) decrease in the percentage of live fetuses and a significant ($p < 0.01$) increase in the percentage of resorbed fetuses. Fetuses had lower body weights and increased incidence of skeletal ossification variants. Thirty-nine percent of the fetuses had either ocular malformations or vertebral defects. At this dose level, MMT demonstrated severe maternal toxicity and embryotoxicity. Mean fetal crown-rump lengths were comparable between the MMT-treated and control groups.

The dose level of 40 mg/kg was highly toxic and led to 100% mortality of the dams within the first 5 days of treatment. No fetuses were recovered for examination. The authors concluded that MMT was not embryotoxic or teratogenic at 5 mg/kg/day but could not assess the teratogenic potential of MMT at either 10 or 20 mg/kg/day.

Majima (1985) administered MMT (10 mg/kg) to six pregnant CF-1 mice on day 12 of gestation. Twenty pregnant females served as controls. MMT had no adverse effects on the number of corpora lutea, implantations, and living fetuses. The concentration of MMT was significantly (p value not given) higher in the livers of the treated dams as compared to the untreated controls, with slight increases (not significant) in the lungs and pancreas.

C. Genotoxicity

SRI (1977, as cited in USEPA, 1983) evaluated the mutagenic potential of MMT in microbial (Salmonella/microsome and Saccharomyces cerevisae) assays both with and without metabolic activation. The S. typhimurium strains employed were TA98, TA100, TA1535, TA1537, and TA1538. MMT was nonmutagenic in all assays.

Bio/dynamics (1977, as cited in USEPA, 1983) tested MMT for mutagenicity in a dominant lethal assay in mice. The compound was administered by gastric intubation to male mice at dose levels of 80 and 160 mg/kg/day for 5 consecutive days. No dominant lethal effects were observed.

D. Structure-Activity Relationships

No compounds that are structurally related to MMT have been selected for toxicological testing by the National Toxicology Program (NTP) (CHEMTRACK, 1986).

IV. Nomination Source

A. Source: Environmental Protection Agency (USEPA, 1984)
B. Recommendation: Three-generation reproductive toxicity studies
C. Rationale/Remarks: Moderate production volume, high skin absorption, and toxic effects have been observed in humans.
D. Priority: None given
E. Date of Nomination: July 1984

V. Chemical Evaluation Committee Review

A. Date of Review: April 29, 1986
B. Recommendations: No testing
C. Priority:
D. NTP Chemical Selection Principle(s):
E. Rationale/Remarks: -Low potential for consumer exposure. Not used in U.S. as a gasoline additive.

VI. Board of Scientific Counselors Review

A. Date of Review: November 25, 1986
B. Recommendations: No testing
C. Priority: —
D. Rationale/Remarks: -Low exposure
-Reconsider if new uses developed for MMT

VII. Executive Committee Review

A. Date of Review:

B. Decision:

VIII. Information Sources

This report was prepared by a multidisciplinary team of scientists and technicians. Dr. John Bruno was the principal author.

The information resources used in preparing this review include the automated data bases listed below, journal articles, general reference materials, and contractor/agency reports.

ON-LINE DATA BASES SEARCHED

MEDLARS

CHEMLINE	
RTECS	
TDB	
MEDLINE	1983-Present
TOXLINE	1966-Present
TOX 76	1976-1980
TOX 65	1940-1975
CANCERLIT	1963-Present
CANCERPROJ	1978-1981

DIALOG

AGRICOLA	1970-Present
AQUALINE	1960-Present
BIOSIS PREVIEWS	1969-Present
CA SEARCH	1967-Present
CHEMICAL EXPOSURE	1974-Present
CIN (Chemical Indust. Notes)	1974-Present
CLAIMS/U.S. PATENT ABSTRACTS	1950-Present
CONFERENCE PAPERS INDEX	1973-Present
CRGS (Chemical Regulations and Guidelines) System)	1982-Present
EMBASE	1974-Present
ENVIROLINE	1971-Present
ENVIRONMENTAL BIBLIOGRAPHY	1974-Present
FEDERAL REGISTER ABSTRACTS	1977-Present
FEDERAL RESEARCH IN PROGRESS	1976-Present

FSTA (Food Science and Technology Abstracts)	1969-Present
GPO	
IPA (International Pharmaceutical Abstracts)	1970-Present
LIFE SCIENCES COLLECTION	1978-Present
METADEX	1966-Present
NTIS	1970-Present
OCCUPATIONAL SAFETY AND HEALTH	1972-Present
PTS PROMT	1972-Present
PTS F&S INDEXES	1972-Present
POLLUTION ABSTRACTS	1970-Present
SCISEARCH	1974-Present
WORLD TEXTILES	1970-Present

CIS

OHMTADS
SPHERE, CESARS, DERMAL, ENVIROFATE, GENETOX, and ISHOW

BRS

KIRK-OTHMER	1978-Present

INFOLINE

LABORATORY HAZARD BULLETIN	1981-Present
CURRENT AWARENESS IN BIOLOGICAL SCIENCES	1983-Present
CHEMICAL HAZARDS IN INDUSTRY	1984-Present
WORLD SURFACE COATING ABSTRACTS	1976-Present

OTHERS

CECATS	
CURRENT AWARENESS	
DIDS	1950-Present
EMIC	1940-Present
ETIC	
EPACASR	
ITS	
NOES	
NOHS	
NTP CHEMTRACK	
STORET	
TSCA INVENTORY	
HAZARDLINE	1983-Present
OSHA MONITORING DATA BASE	

National Occupational Hazard Survey

PROJECTED NUMBERS BY INDUSTRY 01/22/80

CAS # 12108013 3 HAZ 08547 DESCRIPTION METHYLCYCLOPENTADIENYL MANGANESE TRICARBONYL

SIC CODE	DESCRIPTION	ESTIMATED PLANTS	ESTIMATED PEOPLE		ESTIMATED EXPOSURES
28	CHEMICALS AND ALLIED PRODUCTS	129	1,620	**	1,620
29	PETROLEUM AND COAL PRODUCTS	19	641	***	641
30	RUBBER AND PLASTICS PRODUCTS, NEC	8	176	***	176
33	PRIMARY METAL INDUSTRIES	40	2,130	***	2,130
34	FABRICATED METAL PRODUCTS	188	3,768	***	3,768
35	MACHINERY, EXCEPT ELECTRICAL	98	10,963	***	10,963
36	ELECTRICAL EQUIPMENT AND SUPPLIES	27	27	***	27
37	TRANSPORTATION EQUIPMENT	81	1,630	***	1,630
38	INSTRUMENTS AND RELATED PRODUCTS	22	22	***	22
39	MISCELLANEOUS MANUFACTURING INDUSTRIES	23	1,483	**	1,483
46	PIPE LINE TRANSPORTATION	11	34	***	34
53	RETAIL GENERAL MERCHANDISE	79	474	***	474
TOTAL		720	22,967	***	22,967

** USE FIRST STANDARD DEVIATION COLUMN, EMPLOYEE TABLE
*** USE SECOND STANDARD DEVIATION COLUMN, EMPLOYEE TABLE

ENCLOSURE 1
NOHS

National Occupational Hazard Survey

PROJECTED NUMBERS BY OCCUPATION 01/22/86

CAS # 12108133 HAZ 88867 DESCRIPTION METHYLCYCLOPENTADIENYL MANGANESE TRICARBONYL

OCC CODE	DESCRIPTION	ESTIMATED PLANTS	ESTIMATED PEOPLE	ESTIMATED EXPOSURES
035	CHEMISTS	08	114 ***	114
151	CHEMICAL TECHNICIANS	152	432 ***	432
102	ENGINEERING AND SCIENCE TECHNICIANS, N.E.C.	24	47 ***	47
425	DECORATORS AND WINDOW DRESSERS	79	474 **	474
441	FOREMEN, N.E.C.	45	318 ***	318
446	HEAT TREATERS, ANNEALERS, AND TEMPERERS	134	360 **	360
501	MACHINISTS	56	1,077 ***	1,077
502	MACHINIST APPRENTICES	5	97 ***	97
481	HEAVY EQUIPMENT MECHANICS, INCL. DIESEL	5	59 ***	59
546	OPTICIANS, AND LENS GRINDERS AND POLISHERS	22	22 **	22
530	PRESSMEN AND PLATE PRINTERS, PRINTING	4	7 ***	7
505	STATIONARY ENGINEERS	19	75 ***	75
561	TOOL AND DIE MAKERS	89	3,426 ***	3,426
562	TOOL AND DIE MAKER APPRENTICES	30	261 ***	261
602	ASSEMBLERS	35	714 ***	714
610	CHECKERS, EXAMINERS, AND INSPECTORS; MANUFACT	29	275 ***	275
622	FURNACEMEN, SMELTERMEN, AND POURERS	24	919 ***	919
642	OILERS AND GREASERS, EXC. AUTO	14	14 ***	14
643	PACKERS AND WRAPPERS, EXCEPT MEAT AND PRODUCE	57	1,513 ***	1,513
644	PAINTERS, MANUFACTURED ARTICLES	47	94 ***	94
650	DRILL PRESS OPERATIVES	77	5,374 ***	5,374
651	GRINDING MACHINE OPERATIVES	24	238 ***	238
652	LATHE AND MILLING MACHINE OPERATIVES	67	2,696 ***	2,696
656	PUNCH AND STAMPING PRESS OPERATIVES	14	203 ***	203
662	SAWYERS	27	27 ***	27
680	WELDERS AND FLAME-CUTTERS	26	106 ***	106
690	MACHINE OPERATIVES, MISCELLANEOUS SPECIFIED	176	967 ***	967
692	MACHINE OPERATIVES, NOT SPECIFIED	47	1,453 ***	1,453
694	MISCELLANEOUS OPERATIVES	8	176 ***	176
695	NOT SPECIFIED OPERATIVES	26	81 **	81
704	FORK LIFT AND TOW MOTOR OPERATIVES	50	76 ***	76
753	FREIGHT AND MATERIAL HANDLERS	45	555 ***	555
903	JANITORS AND SEXTONS	14	116 ***	116
TOTAL		*	22,967 ***	22,967

* ESTIMATED PLANTS NOT ADDITIVE BY OCCUPATION
** USE FIRST STANDARD DEVIATION COLUMN, EMPLOYEE TABLE
*** USE SECOND STANDARD DEVIATION COLUMN, EMPLOYEE TABLE

ENCLOSURE 2
NOHS

NATIONAL OCCUPATIONAL EXPOSURE SURVEY AS OF: 01/23/80 PAGE 4

ESTIMATED TOTAL AND FEMALE EMPLOYEES
FIELD OBSERVATION DATA

CAS # NIOSH # HAZ DESCRIPTION

0P0121000133 OP1950000 00597 MANGANESE, TRICARBONYL(METHYL-PI-CYCLOPENTADIENYL)-

SIC CODE	DESCRIPTION	PLANTS	TOTAL EMPLYS	FEMALE EMPLYS	TOTAL EXPOS	FEMALE EXPOS
29	PETROLEUM AND COAL PRODUCTS	10	1,000		1,000	
TOTAL		10	1,000		1,000	

NATIONAL OCCUPATIONAL EXPOSURE SURVEY AS OF: 01/23/80 PAGE 4

ESTIMATED TOTAL AND FEMALE EMPLOYEES
FIELD OBSERVATION DATA

CAS # NIOSH # HAZ DESCRIPTION

0P0121000133 OP1950000 00597 MANGANESE, TRICARBONYL(METHYL-PI-CYCLOPENTADIENYL)-

OCC CODE	DESCRIPTION	PLANTS	TOTAL EMPLYS	FEMALE EMPLYS	TOTAL EXPOS	FEMALE EXPOS
453	JANITORS AND CLEANERS	10	605		605	
754	PACKAGING AND FILLING MACHINE OPERATORS	10	68		68	
856	INDUSTRIAL TRUCK AND TRACTOR EQUIPMENT OPERATORS	10	215		215	
869	LABORERS, EXCEPT CONSTRUCTION	10	90		90	
999	NO OCC CODE AVAILABLE	10	20		20	
TOTAL			1,000		1,000	

ENCLOSURE 3
NOBS

National Occupational Hazard Survey

NIOSH TRADENAME INGREDIENT DATA BASE - NOHS DATE 11/22/85 PAGE 53

84547 METHYLCYCLOPENTADIENYL MANGANESE TRICARBONYL

0219879 ETHYL CORP PO BOX 341 BATON ROUGE, LA 70821

0219878 COMBUSTION IMPROVER NO. 2 96 %

IX. References

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Clinical Manganism and Exposure to Manganese in the Production and Processing of Ferromanganese Alloy

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In 1963 the American Conference of Governmental Industrial Hygienists (ACGIH) changed the threshold limit value* (TLV) for manganese so that 5 milligrams of manganese per cubic meter of air (mg/cu m) represented a Ceiling Value.† In 1965, following the lead of the Threshold Limits Committee, the Pennsylvania Department of Health, under the Regulations Establishing Threshold Limits in Places of Employment adopted a Short Term Limit (STL) for manganese of 5 mg/cu m for 30 minutes.

As a consequence of those changes the medical and environmental quality control personnel of a large ferromanganese producer in Pennsylvania felt that a reappraisal of the environment and the health of employees working in ferromanganese production and processing facilities was indicated.

Manganism and Parkinsonism

The syndrome of manganism is that of Parkinson's disease and is clinically indistinguishable from it. A review of the literature elicits only about 400 cases of manganese intoxication since it was first reported over 100 years ago. Most of these cases occurred following exposure to manganese dioxide ore or to manganese dioxide fume produced by oxidizing processes such as cutting or burning.

Parkinsonism is a progressive neurological disease which results from destruction of the cells of the basal ganglia. Occasionally, it is seen as an acute illness following hemorrhage of the brain. Rarely, it is seen in association with brain tumor or syphilis. Arteriosclerotic, rather than primary degenerative changes, can occasionally be a cause of the shaking palsy of very advanced age. Until 1915, parkinsonism was mainly a disease of advanced age, rarely occurring before 40 and usually setting in between 50 and 60 years of age.

Poskanzer and Schwab (Massachusetts General Hospital, 1961) feel that most of the parkinsonism now seen is the result of the worldwide epidemic of encephalitis lethargica that lasted from 1915 to 1926. They expected that the number of cases would increase for some 40 years after the epidemic and then dwindle as the victims died from this or other causes. In 1961 the mean age of persons newly afflicted with parkinsonism was 60.6 compared with 34.7 in 1932. Poskanzer and Schwab subsequently examined 421 additional patients and found that none of them were born after 1931, the year the virus died out.

Cotzias[1] was the first to demonstrate a therapeutic response to high doses of levodopa. The rationale for its use had developed from studies showing that dopamine normally occurs in high concentration in the basal ganglia and substantia nigra, but at autopsy it was markedly depleted in those areas of the brains of persons having parkinsonism. Since dopamine does not cross the blood-brain barrier, its administration is ineffective. However, levodopa, the metabolic precursor of dopamine, does cross the blood barrier and presumably is converted into dopamine in the basal ganglia.

It is the opinion of some investigators (Penalver R, oral communication, and Whitlock et al[6]) that the parkinsonism syndrome produced by manganism is not progressive if the victim is promptly removed from exposure, and that the condition may improve following the use of chelating agents. Penalver has shown moving pictures demonstrating improvement in one case by simply removing the patient from further exposure, chelating drugs being not yet available.

Massive exposure to the fume or dust of manganese may rapidly produce symptoms and signs at any age in contrast to the slow and insidious course of parkinsonism due to other etiology.

The main portal of entry of manganese is the respiratory tract. The gastrointestinal tract plays little or no role and radioisotopic studies reveal that inorganic manganese salts are slowly and poorly absorbed by this route. The main route of excretion is in the bile, the liver being the preferred site of accumulation. Only small amounts of manganese are found in the urine, even following heavy exposure. Chelating agents, however, markedly increase the excretion by the kidneys. Manganese is an essential element for normal metabolism and with an average daily intake of 3 to 9 mg, the blood contains from 12 to 15 μg/100 ml and the urine

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somewhat less than 10 μg/liter.[2] Most of the intake is eventually accounted for in the feces. Maynard and Cotzias[3] demonstrated that manganese disappeared rapidly from the blood and was concentrated in the liver, kidneys, and brain.

Production and Processing Methods

From 1923 to the present, the production facility for ferromanganese at the operation under study has been the blast furnace. There have been changes, however, in the handling of the casted material. From 1923 to 1957, the ferroalloy was poured from the furnace into cast-cars (low-sided gondola railroad cars lined with refractive material). The casted material was removed from the cast-car and sized and sorted manually. In 1957, due to customer demand for smaller and more uniform sizes of the alloy, crushing and screening facilities were installed in the then existing building. In 1961, a new and larger building was erected to house crushing and screening equipment of much greater capacity. In December 1961, the molten ferromanganese was tapped from the blast furnace into conventional submarine ladles and transported to a pig-casting machine.

Nature of Exposure

Dust Exposure. — The mechanized crushing and screening operation resulted in the production of ferromanganese dust. Visual observation indicated that the overall dustiness associated with mechanical crushing and screening was greater than that associated with manual operations. Prior to 1957 dust was not generally recognized as being a hazard although Patty[2] stresses the mining, transporting, crushing, and sieving of the manganese ore as being the chief source of exposure and Voss[4] had reported one case of manganese intoxication in a man milling and grinding ferromanganese.

Dust sampling studies were therefore begun in the processing plant shortly after the commencement of crushing and screening operations in November 1957. At that time, the processing facilities were housed in a three-sided building measuring 335 ft x 106 ft (Fig 1). The south end of the building was completely open and there were large openings along both the east and west walls. On the west side close to the north wall, ferromanganese, which had been dug out of the cast-cars by Gradalls (an unloading machine with a claw-like attachment at the end of a telescopic boom), was loaded into boxes for charging into a jaw-type crusher. The crusher and screens were located at the north end of the building midway between the east and west sides. The finished product left the plant by truck through the large opening in the east wall and by rail from the open, south end.

Table 1. — Manganese-in-Air Dust Concentrations Before Ventilation — Old Preparation Plant

Type and Location of Sample	Number of Samples	Concentration (mg/cu m)*		
		High	Low	Average
Breathing Zone				
Crusher Operator and Helper	6	80.0	8.2	35.0
General Air				
Screening Station	6	52.0	9.0	27.0
Crusher Discharge	3	1750.0	405.0	1122.0
Screened Material	5	350.0	27.0	119.0

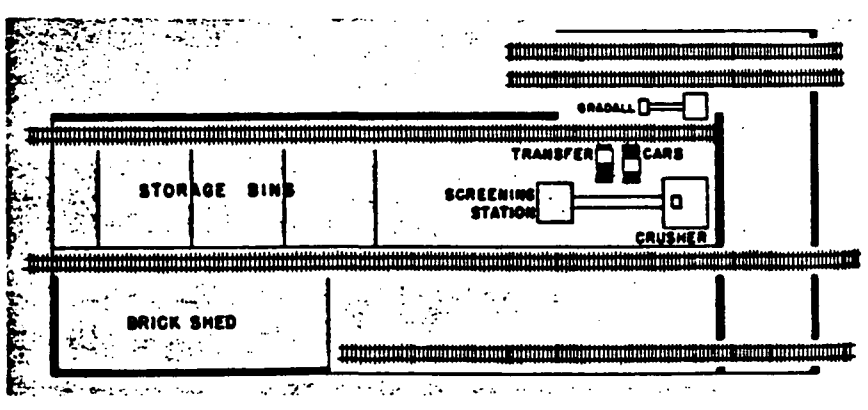
* Milligrams per cubic meter of air.

Because of the physical layout of the building and the working pattern of the employees, the midget impinger was used. (There were only two men, the Crusher Operator and Helper, both of whom remained fairly stationary on a platform atop the crusher.) Twenty samples, each of 15-minutes duration, were obtained over a two-day period in January 1958. The findings are listed in Table 1. Manganese-in-air concentrations averaged 1122 mg/cu m in the area immediately around the crusher discharge, which was the major source of dust. No employee was stationed in this area. Much lower concentrations (average 27 to 35 mg/cu m) were found in those areas in which employees were stationed, namely, on the platform atop the crusher and at the screening area. The time that employees remained in those areas of excessive manganese dust was not measured. Time-weight averages of manganese exposure, although obviously lower than the numbers cited, were also not determined.

At that time the TLV for manganese was 6 mg/cu m. Inasmuch as the dust measured concentrations were greatly in excess of that level, it was recommended that local exhaust ventilation be designed for all dust sources and installed as soon as possible. Until the ventilation system was operable, a respiratory protective program was made mandatory and initiated immediately.

In May 1959 an attempt was made to establish a procedure for monitoring the dust concentrations to which the employees in the Processing Plant were exposed. The felt filter pads were collected from the face pieces of the respirators after an eight-hour turn had been worked by the Crusher Operator and Helper. Filters from eight successive work shifts were obtained for each position. The filters were analyzed quantitatively for manganese. By assuming that a workman inspires approximately ten cubic

Fig 1. — Plan view of old manganese processing plant (building dimensions 335 ft by 106 ft).



meters of air over an eight-hour work day, the exposures to manganese dust concentrations were calculated to be 47 mg/cu m for the Crusher Operator and 52 mg/cu m for the Helper. These findings were comparable to the 35 mg/cu m as determined previously by air sampling conducted in the breathing zone of the Crusher Operator and Helper. If one could with certainty know that the exposed employee was faithful in wearing his respirator, this procedure could be a useful method of monitoring the exposure. Because such assurance could not be given without constant supervision and because exhaust ventilation subsequently reduced the dust concentrations to acceptable limits, the procedure was abandoned.

Despite the installation of the crusher and rudimentary screening operation, some manual sizing with sledge hammers was still required. This work was performed near the open, south end of the building by four to six laborers. The laborers' total exposure to the dust created by their own work and by the crushing and screening equipment was determined by use of an electrostatic precipitator. Eighteen tests, each of approximately one-hour duration, were obtained over six different days in May 1959. The concentration of all 18 tests averaged 5 mg/cu m. Since the tests were conducted only during the time of processing and not during repair or lunch periods, the weighted-average concentrations would have been somewhat lower than 5 mg/cu m. Inasmuch as the measured concentrations were below the TLV of that time, the wearing of respirators was placed on a voluntary rather than a mandatory basis in that area of the building.

The installation of the exhaust ventilation system was completed in February 1960. During March 1960, in order to evaluate the efficiency of the new system, air samples were collected with a midget impinger and an electrostatic precipitator on the platform atop the crusher and in other work areas around the crusher and screens. The manganese dust concentrations averaged 0.7 mg/cu m atop the crusher and 0.6 mg/cu m in the adjacent work areas; the highest concentration was only 1.84 mg/cu m. This was a dramatic improvement over the excessively high levels measured prior to the installation of the exhaust system.

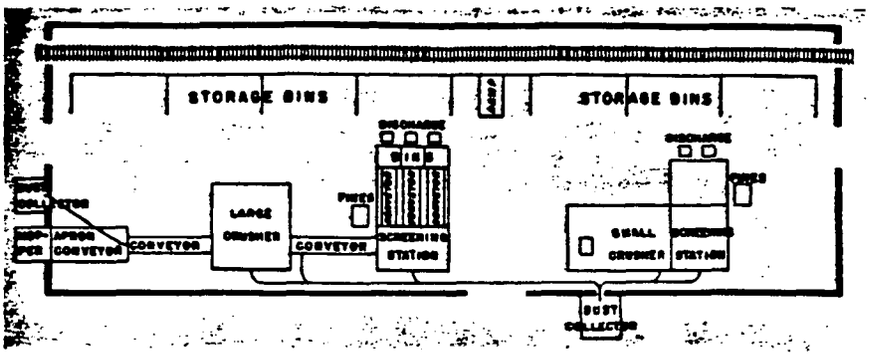


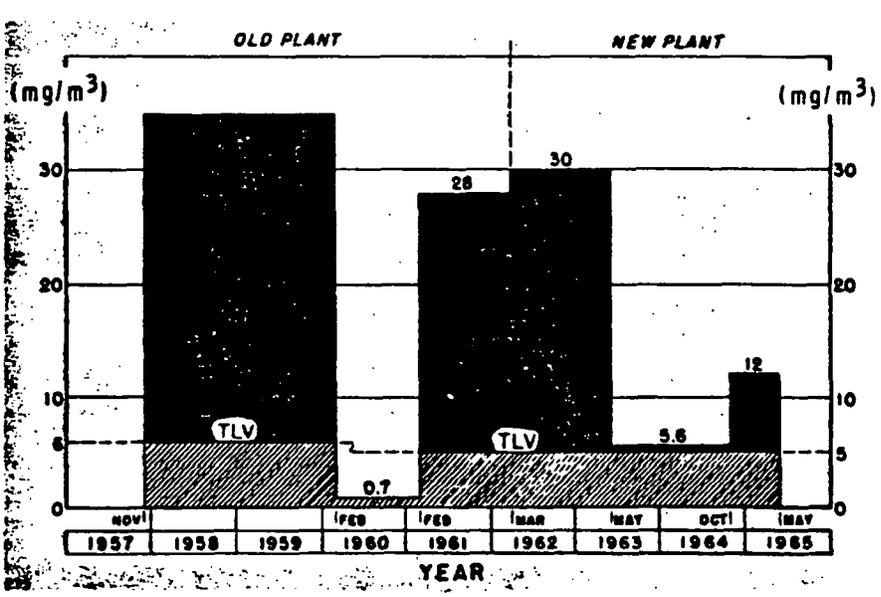
Fig 2. — Plan view of new manganese processing plant (building dimensions 400 ft by 128 ft).

Because of the extreme abrasiveness of the dust, however, the ductwork in the ventilation system soon began to erode, particularly in the elbows, where holes began to appear. In addition, because of the bulkiness of the large and heavy loading boxes which required frequent moving, inadvertent damage to the hoods ensued. Both these factors contributed to a gradual reduction of the air volume needed to satisfactorily transport the contaminant from the work areas. Further atmospheric testing to determine exposure to manganese dust was conducted during January and February of 1961. Ten tests in the breathing zone of the Crusher Operator and the Bottom Man (employee working primarily in the area around the screening equipment, who was responsible for seeing that sized material from screening operations was properly loaded and removed for shipping) were obtained with a midget impinger over four different days. The average dust concentration immediately following installment of the ventilating system was 3.2 mg/cu m for the Crusher Operator and Helper (about a fivefold increase over that of the 1960 study), and 53 mg/cu m for the Bottom Man. Because of these relatively high dust concentrations, management was urged to make every effort to maintain the ductwork and hoods and require the wearing of respiratory protective devices when excessive dust concentrations were encountered.

Because customers demanded a wider range of small sizes of the alloy as well as varied manganese composition (range 74% to 82%), it was necessary to purchase another crusher, smaller in size than the original, but of the same type. It was also decided to house the crushing and screening equipment in a new and larger processing building (Fig 2). By March 1962 the changeover was completed. At the same time the old "large" crusher and screens were supplemented with the new "small" crusher and its auxiliary equipment.

In the new processing building an attempt was made to use water sprays as a

Fig 3. — Summary of average ferromanganese dust concentrations.



dust suppressant at material transfer points in lieu of hoods and exhaust ventilation. It was apparent from the start that sprays, while accounting for some reduction, were not lowering the dust concentrations to satisfactory levels. To confirm these observations, general air samples were obtained with an electrostatic precipitator during June and August 1962. Concentrations during this study ranged between 12 and 83 mg/cu m and averaged 30 mg/cu m. Based on these tests it was recommended that water spraying be discontinued and an exhaust ventilation system be installed. In May 1963 the exhaust ventilation system was completed for both the large and small crushers as well as their respective screening equipment.

Sampling of the air-borne contaminant by midget impingers to determine the effectiveness of the new ventilation system was completed by October 1964. Twenty-five general air samples, each of 10- to 12-minutes duration, were taken over the various working locations during the survey period.

On five of the nine test dates, the average daily concentration exceeded the TLV for manganese; of the 25 samples, nine exceeded 5 mg/cu m. However the average concentration (5.6 mg/cu m) of all the samples was just slightly above the TLV.

Fig 3 shows that between November 1959 and May 1965 average ferromanganese dust concentrations in the processing plant were in excess of the TLV for much of the time.

The processing plant operation requires periods of down-time during the course of a work day because of the necessity to change screens for the varied sizes produced and for crusher malfunction. The average concentrations expressed in Fig 3 reflect both periods of down-time as well as periods of peak production. In spite of that, it is apparent that for appreciable periods of time over the 7 1/2-year period (1957 to 1965) employees were exposed to higher than desirable levels of manganese and during the periods when local exhaust ventilation was not operative, average concentrations were three to six times the TLV.

Fume Exposure. — At this operation, ferromanganese is produced in a blast furnace. Ferromanganese fume is emitted during the 20 to 30 minutes of the cast as a dense, orange plume which arises from the trough running from the blast furnace to the ladle and slag pots and spreads rapidly over the cast house floor. Because of the natural movement of air across the cast house floor, the plume is rapidly dissipated following the conclusion of the cast.

The First Helper experiences the highest exposure due to the fact that his work requires him to maintain a position relatively close to the trough during the entire cast. Other employees are able to remain in the Blowers Shanty (enclosed control room) or at the furthest upwind portion of the cast house away from the most dense portion of the plume.

The exposure at the pig-casting machine is also to ferromanganese fume arising from the runner leading from the ladle to the pig moulds. The exposure lasts for about 1 1/2 to 2 hours per pouring operation (5 times per 24 hour period).

The Pourer's exposure is greater than that of the Tilter because of his working position in relationship to the runners conveying the molten metal. His work requires him to maintain the runner (clearing slag, etc) in close proximity to the source of the fume, whereas, the Tilter is in an enclosed pulpit and at some distance from the plume.

In early 1964 a significant event which occurred in a plant in central Pennsylvania was brought to our attention.[6] Two men employed to cut and trim manganese steel castings with air-arc burners in a small, unventilated enclosure were admitted to a Harrisburg, Pa, hospital with symptoms and signs of central nervous system impairment manifested by progressive weakness, unsteadiness of gait, loss of co-ordinated movements and increasingly severe hoarseness of the voice. There was marked loss of strength in all extremities, abnormal reflex changes, intention tremor and a sense of euphoric, carefree attitude not previously characteristic of the personality of the employees. An inexpressive facial masking was present as well.

Following EDTA† treatment, there was an immediate high excretion of urinary manganese (as much as 1,000 µg/liter in one patient) and, with the passage of time, an improvement in the clinical appearance of the employees. It was concluded by the authors that the TLV for manganese-in-air concentrations provided little or no factor of safety, thus confirming the opinion expressed by the ACGIH Threshold Limits Committee when a ceiling value was established. In effect, this event indicated to the investigators that serious manganese intoxication might occur when weighted manganese-in-air concentrations were below the TLV as then defined: 5 mg/cu m (ceiling value).

Our attention was now directed to production of ferromanganese at the operation being studied because of the recognized manganese fume exposure which we believed might be similar to that of the reported cases in the Harrisburg area. In February 1966 it was decided to perform an epidemiological study of the entire problem.

Environmental Phase

Method of Study. — Fifteen positions, as indicated by previous environmental sampling and observation to have the highest exposures, were to be studied; nine at the production (fume) area and six at the processing (dust) area. Since the Pennsylvania STL for manganese is based on a 30-minute period, all air samples throughout the study were collected for this length of time. All air samples were taken in the worker's breathing zone and sampling was done in all four seasons of the year.

Fume. — For the fume exposures the electrostatic precipitator was used as the basic sampling instrument. That instrument, however, requires the investigator to hold the sampler in the vicinity of the worker's breathing zone. Since some job positions, such as the First Helper on the Cast-House floor, were too hazardous to permit that procedure, the electrostatic precipitator was supplemented with the millipore filter and monitaire pump which could be mounted on the employees.

Before the study was begun, simultaneous sampling with the electrostatic precipitator and millipore filters was performed and it was determined that results obtained from the two methods were comparable.

The survey of the nine job positions at the production facilities consisted of collecting air samples during the casting of the furnace and during the pigging operations. Additional samples were obtained before and after casting and pigging to determine concentrations to which the employees were exposed

during the intervals between the times of fume production.

Dust. — For the dust exposures, sampling was done with the midget impinger, the collecting flask being suspended from the worker's shirt. The monitaire pump was the vacuum source. Early in the study it was determined that the midget impinger samples were being contaminated. Millipore filters, which were difficult to inadvertently contaminate, were substituted for impinger flasks. Testing showed that the results obtained by the two sampling techniques were comparable and the millipore filter sampling method was used for the duration of the study.

In the Preparation Plant, where the ferromanganese is processed almost continuously, samples were collected over the entire three workshifts for the six positions being studied.

Results

Fume. — Table 2 summarizes the results of the breathing zone samples by position at the Blast Furnace and Pig Caster. The number of samples and the range as well as the weighted-average concentrations for each job position are shown. In addition, the number and percent of samples exceeding the short-term limit are listed.

At the Cast-House none of the job position exposures exceeded the TLV of 5 mg/cu m. The highest weighted-average concentration (3.6 mg/cu m) was found for the First Helper and the lowest (0.12 mg/cu m) for the Cinderman. On the other hand, the short-term limit was exceeded on numerous occasions at six of the seven positions.

At the Pigging Machine, the Pourer's exposure (13.3 mg/cu m) was in excess of the TLV, whereas, that of the Tilter was slightly below (4.4 mg/cu m). However, 93% of the samples for the Pourer and 75% of the samples for the Tilter exceeded the STL.

Fig 4 shows the particle size distribution of the air-borne fume. One hundred percent of the fume is composed essentially of manganese oxide, but mainly Mn_3O_4, and is less than 2μ in size, all of which is respirable.

Dust. — At the Crushing and Screening Plant, as is seen in Table 3, the TLV was exceeded at three of the six positions studied. Those included the Screen Plant Helper (12.9 mg/cu m), Screen Plant Operator (6.3 mg/cu m) and Fork-Lift Operator (5.2 mg/cu m). As was the case in the Production Facility many of the samples were in excess of the STL.

Table 2. — Manganese Fume-in-Air Concentrations by Position

Position	Number of Samples	Samples Exceeding Short Term Limit (STL)		Concentration (mg/cu m)		
		Number	% of Total	Low	High	Weighted-Average
Blast Furnace Cast House						
Keeper	35	5	31.00	0.04	39.5	1.86
Slower	26	5	31.00	0.04	39.5	1.34
Hot Blastman	11	1	9.33	0.02	5.9	0.33
Turn Repair Pipework	6	1	17.00	0.05	12.7	1.28
Second Helper	9	2	22.00	0.04	9.6	0.51
First Helper	10	7	70.00	2.20	171.0	3.60
Cinderman	8	0	0.00	0.02	1.9	0.12
Pig Casting Machine						
Tilter	24	18	75.00	0.02	43.9	4.40
Pourer	14	13	93.00	3.20	206.0	13.30

Particle size distribution of the airborne dust was determined and is plotted in Fig 5. Ninety-five percent of the dust is less than 5 μ in size, and is of respirable size.

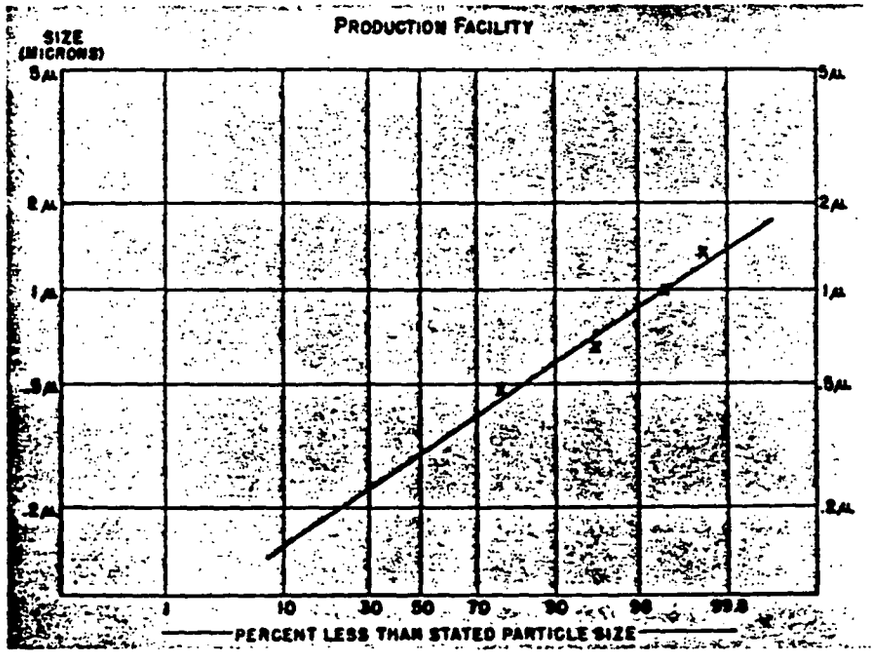
The dust was analyzed for its chemical composition by x-ray diffraction and infra-red methods. Results showed that the metallic content of the dust was mainly ferromanganese (FeMn) with small amounts of manganosite (MnO), Hausmannite (Mn_3O_4) and iron oxide (Fe_2O_3).

Clinical Phase

Method of Study. — It was decided that a study of 71 employees, exposed daily for eight-hour shifts in three areas involving 15 positions, would render the greatest information. A second group of 71 male employees was selected for identical study. These men, chosen from the plant generally and never having been directly exposed to manganese, were matched for age, length of plant service, and ethnic background.

The routine procedures on all persons studied included a history, physical examination, and certain laboratory examinations. All employees received an examination by a neurologist. Blood slides for stipple cell counts were examined by a pathologist. Urine and blood manganese assays were performed using a method especially developed for this purpose.[7] The neurological examination, stipple counts, and blood and urine assays were

Fig 4. — Particle size distribution of airborne fume (production facility).



performed as blind studies to insure objectivity. Additional laboratory studies included chest x-ray, red blood cell count, white blood cell count with differential, hematocrit reading, blood sugar, sedimentation rate, urine analysis, and electrocardiogram.

Results. — Examination of the 142 employees elicited five cases with symptoms and signs of central nervous system impairment highly suggestive of manganism. All five were from the exposed group and all but one occurred in positions of highest manganese exposure. Three were exposed to manganese fume and two were exposed to dust.

The salient features of each case are listed below.

Case 1. Age 47. Twenty-three years intermittent exposure to ferromanganese dust as an Unloader, Screen Plant Operator, and Helper. When examined was found to have had history of parkinsonism for five years. Clinically he exhibited marked masking of the facies, loss of blinking reflex, absence of associated movements of the left arm when walking, tremor of left arm and head, and cogwheel rigidity of left arm and leg. There was micrographia.

Case 2. Age 48. Twenty-three years intermittent exposure to ferromanganese dust as an Unloader, Screen Plant Operator, and Helper. When examined he had no complaints but on neurological examination he exhibited complete loss of associated arm movements bilaterally.

Case 3. Age 52. Eight-years exposure to manganese oxide fumes, three years as a Laborer in the general blast furnace area, and five years as an Iron Pourer at the Pig Casting Machine. Clinically he had no complaints, but on neurological examination exhibited complete loss of associated arm movements bilaterally.

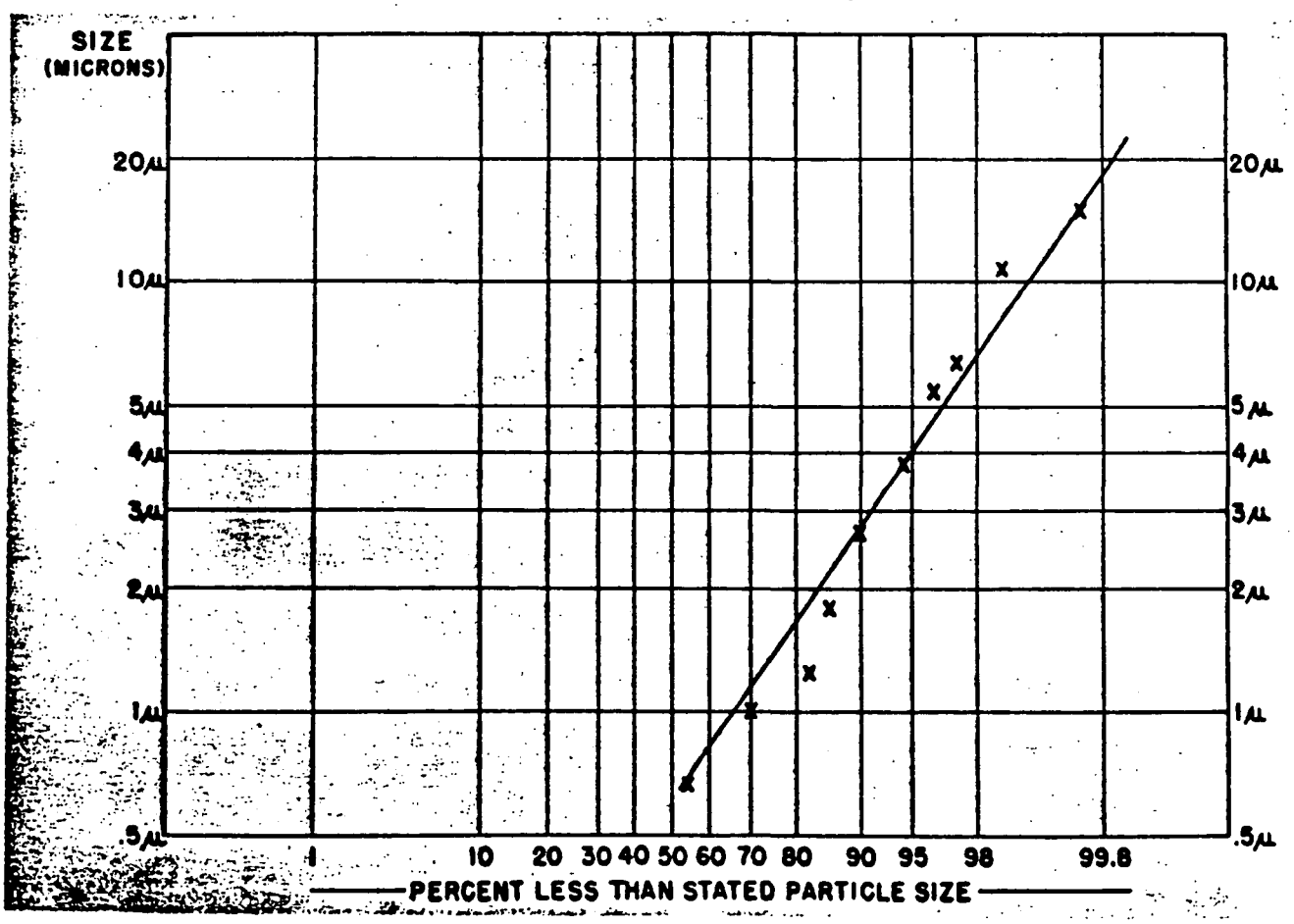
Case 4. Age 49. Twenty-six years intermittent exposure to manganese oxide fumes as a Laborer and Iron Pourer at the Ferromanganese Pig Casting Machine. Clinically exhibited marked masking of the facies, diminished blinking of the eyelids, absence of associated arm movements, muscle rigidity in right arm and both legs. Micrographia was present.

Case 5. Age 49. Ten and one-half years exposure to manganese oxide fumes as a Hot Blastman at the Ferromanganese Blast Furnace. Clinically exhibited micrographia, loss of associated arm movements on the right, and static tremor of the right hand. There was facial masking, reduced blinking reflex, and some cogwheel rigidity in the right arm and leg.

None of the five employees admitted to early complaints of languor, lassitude, or apathy. All five were actively working and one (Case 2) lived on a farm and was productively working it in his spare time. There was no noticeable difficulty in walking or speech impairment. There was no history of muscle cramps, headache, loss of sleep, or excessive sweating as have been reported. Case 5 exhibited excessive salivation and a tendency to drool. No pathologic reflexes were present. None of the employees remembered having been told by family members that they have been influenza victims in infancy or childhood. Neither had any suffered from pneumonia since being employed. (Pneumonitis has been reported as a manifestation of manganism.)

In reviewing the sick absence records for the five employees, it was noted that Case 2 had been ill from January 24,

Fig 5. — Particle size distribution of airborne dust (manganese shed).



1952, to July 13, 1952 (182 days), with a diagnosis of "Psychosis" which we believe may have been representative of a transient personality change. When questioned about the episode, the employee stated he "trembled" when he tried to perform fine mechanical tasks such as threading a nut on a bolt and he would "break out into a sweat." However, the nature of the incident is not clear and its significance unknown. The personality of the employee at the time of examination was "normal" in every respect. Nevertheless, the episode did occur during a period of exposure to ferromanganese dust.

The five employees were hospitalized for further study under the care of an internist. Results of skull films, electroencephalograms, spinal taps, and blood serology were negative for all. Serum iron and serum iron binding capacities were normal. Red blood cell counts, hemoglobin values, white blood cell and differential counts were within normal ranges. Case 2 revealed fine, delicate stippling in a very occasional red blood cell. Case 3 revealed a hypertension with left ventricular hypertrophy and a history of familial hypertension.

Upon hospital admission, collection of 24-hour urine specimens were begun and continued for ten days in Cases 1, 3, and 4; four days in Case 2; and three days in Case 5. The average urine manganese concentrations for the five cases are shown in Fig 6. Except for Case 5, the average manganese concentrations were below 10 μg/liter.

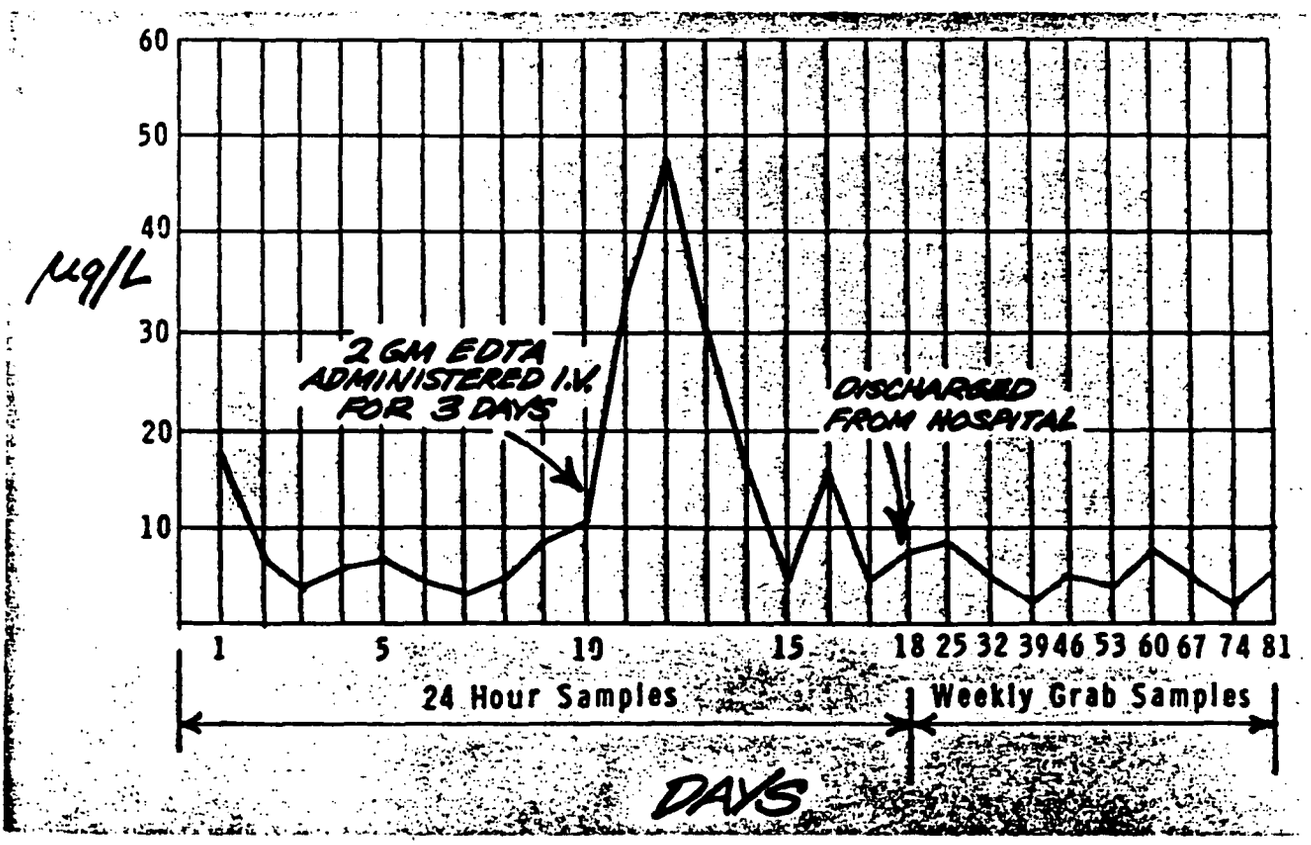
Each hospitalized employee was administered 2 gm of Calcium EDTA in 500 ml of dextrose intravenously on three successive days. Twenty-four hour urine collections were assayed for manganese. The average concentration of manganese in urine before, during, and after treatment is shown in Fig 6. The concentrations do not approach those reported in the Harrisburg cases. Nevertheless, the increased excretion certainly confirms manganese exposure and absorption and indicates mobilization following administration of the chelating agent.

Following discharge from the hospital and release for work in manganese-free areas, spot urine samples were collected weekly for ten weeks. Fig 6 also shows the manganese concentrations during this time. A second course of 2 gm of Calcium EDTA was given, on an ambulatory basis, to Cases 1, 2, and 4. There was little if any increased excretion of manganese. Subsequent weekly spot samples of urine have averaged below 6 μg/liter in all cases.

Three months after the time of the initial neurological examinations all five employees were re-examined. Cases 2 and 3 regained normal associated arm movements. No changes were noted in Cases 1, 4, and 5, and all continued to manifest the same degree of neurological deficit present on the initial examination. While their condition had not improved neither had it deteriorated. Improvement was noted in cases exposed to either manganese fume or ferromanganese dust but only when the neurological deficit did not exceed loss of associated arm movements.

In comparing the exposed and unexposed groups, no significant differences were noted in red blood cell counts, white blood cell and differential counts, hemoglobin levels, and hematocrits. There were seven instances of a history of pneumonia in the unexposed group as compared with eight in the exposed group. Employees having had pneumonia were not in the areas of highest exposure. Sick absence records also indicated no significant difference in the rate of sick absence for the two groups. In the exposed group there were no recorded absences for neurological

Fig 6. — Average urine manganese concentration in five patients—before and after EDTA.



problems except for Case 2.

Stippling of red blood cells has been reported as being a finding in manganism.[8] Blood smears of all employees were examined by a pathologist as a blind study. He reported two types of stippling: a coarse, granular type (reported as positive when one stipple cell was seen in ten high-power fields), and a fine, delicate stippling (reported as positive when seen as an occasional cell per slide). Table 4 reveals no significant difference in the number of persons in the two groups who demonstrated either type of stippling.

Table 5 shows manganese-in-urine concentrations by work area averaged 19 μg/liter in the Processing Plant, 6 μg/liter at the Blast Furnace Cast Floor, and 8 μg/liter at the Pig Casting Machine. The unexposed group averaged 7 μg/liter.

Table 5 further shows that the average manganese blood level of all exposed employees was the same as that of the exposed group, namely 3 μg/100 ml.

Discussion

Several aspects of this study are worthy of comment. Those cases which responded favorably to EDTA treatment (2 and 3) regained normal associated arm movements, the loss of which constituted the only neurological deficit. It is suggested that this sign is a reversible rather than a permanent change, and could be of great importance from the standpoint of early detection and removal from exposure before the occurrence of irreversible central nervous system damage.

The lack of progression of the syndrome over six years lends support to the diagnosis of manganism, since parkinsonism of other etiology is a progressive disease.

The urine manganese concentrations averaged about three times higher in the dust-exposed group than in the unexposed group. While this appears to be a significant difference, it must be remembered that Cholak[9] reported urine concentrations as high as 600 μg/liter in persons with manganese exposure but without clinical evidence of manganism. Further, although the exposure of the Screen Plant Helper was twice that of the Operator, the urine concentration for the Helper was less than half that of the Operator. An attempt to correlate urine manganese concentrations with air con-

Table 3. — Manganese Dust-in-Air Concentrations by Position

Position	Number of Samples	Samples Exceeding Short Term Limit (STL)		Concentration (mg/cu m)		
		Number	% of Total	Low	High	Weighted-Average
Processing Plant						
Screen Plant Operator	51	29	57	0.98	34.7	6.3
Screen Plant Helper	53	37	70	0.88	61.5	12.9
Fork-Lift Operator	30	11	37	0.51	25.9	5.2
Payloader Operator	31	3	10	0.35	5.0	2.1
Craneman	52	9	17	0.18	47.7	4.4
Foreman	30	1	3	0.27	16.2	2.1

Table 4. — Summary of Stippling Observations

	Exposed Group	Unexposed Group
Coarse Stippling (1/10 HPF's)	6 out of 71	9 out of 71
Fine Stippling (Rare/Slide)	9 out of 71	2 out of 71
Total	15 out of 71	11 out of 71

Table 5. — Manganese Blood and Urine Concentrations vs Air Concentrations by Position

Position	Weighted-Average Air Concentration (mg/cu m)		Blood Level (μg/100 gm)	Urine Level (μg/liter)
Blast Furnace				
Keeper	1.1		4	6
Blower	1.3		6	7
Hot Blastman	0.3		2	3
Turn Repair Pipework	1.3		2	10
Second Helper	0.5		2	8
First Helper	3.6		3	7
Cinderman	0.1		2	4
		Average	3	6
Pig Machine				
Tiller	4.4		2	6
Pourer	13.3		3	10
		Average	3	8
Processing Plant				
Screen Plant Operator	6.3		4	53
Screen Plant Helper	12.9		5	21
Fork-Lift Operator	5.2		3	8
Payloader Operator	2.1		5	9
Craneman	4.4		5	12
Foreman	2.1		2	4
		Average	4	19
Control Group		Average	3	7

centrations for each job position resulted in a correlation coefficient of 0.12. Thus there is very poor correlation between manganese exposure and manganese excretion in the urine. In Case 5, the Hot Blastman position, the manganese-in-air concentration was the second lowest exposure measured, yet the urine excretion of manganese (24-hour sample) was the only one to exceed normal prior to EDTA administration. Cholak[9] also states that the mean average for a group of unexposed individuals was 2 μg/liter with a range of 1 μg/liter to 7 μg/liter. The higher mean in our unexposed group (7 μg/liter) may be due to the fact that all steel workers in this plant may be exposed to slightly higher background levels than the normal population. Background atmospheric manganese concentration levels in the areas where the control group was located range from .001 to .025 mg/cu m. Finally, it must be recognized that fundamentally the bile is the primary metabolic pathway for excretion of manganese and the urine cannot very well be expected to reflect occupational exposure accurately. This is doubly apparent when we see manganism develop in the presence of normal urine manganese concentrations.

Equally lacking in correlation are blood manganese concentrations and exposure. The average manganese blood level of our exposed employees was the same as that of the unexposed group.

The development of manganism in one employee having a low exposure to manganese oxide fume (Case 5) suggests the possibility of hypersusceptibility of certain individuals inasmuch as others in similar positions and with equal or greater exposure did not develop manganism. This phenomenon is well known in toxicology. Unknown and unrecognized exposure away from the job site is another possibility. In any event, this case lends stress to the value of periodic physical examinations rather than examinations of biologic materials to detect early evidence of signs of manganism.

Conclusion

Although parkinsonism and manganism are indistinguishable, the detection of five cases among persons having an occupational manganese exposure, and in an age group in which parkinsonism is not expected, justifies the presumption of a causal relationship. The mobilization and excretion of large amounts of stored manganese after treatment with Calcium EDTA supports this presumption.

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*Threshold Limit Value: The maximum, average, atmospheric concentration of contaminant to which workers may be exposed for an eight-hour working day.

†Ceiling Value: A calculated value which may not be exceeded at any time; usually assigned to substances having a threshold limit value with little or no safety factor.

‡EDTA: Calcium Ethylene Diamine Tetra-acetic Acid. A chelating agent chemically binding the manganese into a form excretable by the kidneys.

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METHYLCYCLOPENTADIENYL MANGANESE TRICARBONYL (MMT) IN PETROL: THE TOXICOLOGICAL ISSUES

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ABSTRACT

Methylcyclopentadienyl manganese tricarbonyl (MMT), when used as an octane improver in petrol, leads to increased airborne levels of manganese in the form of Mn_3O_4. The potential health effects of increased airborne manganese are considered in this paper. Manganese, unlike lead which it can replace in petrol, is a normal and essential component of the human diet and the intake from airborne manganese is slight by comparison to the normal dietary intake. The major toxicological effects of manganese, observed after long occupational exposure, are on the lung (manganese pneumonia) and the central nervous system (manganism). The small increase in airborne manganese from the use of MMT in petrol is 3–4 orders of magnitude lower than the level required to produce toxic symptoms of manganese exposure, even in areas of high traffic density, and no health risk from the use of MMT is likely.

INTRODUCTION

Methylcyclopentadienyl manganese tricarbonyl (MMT) can be used as an octane improver in unleaded or leaded petrol. In the internal combustion engine of cars, it is converted into inorganic oxides of manganese, the principle one being manganous manganic oxide ($Mn^{II}Mn_2^{III}O_4$ or Mn_3O_4), which is the major product. Manganese has long been considered a health hazard at the high levels of exposure often found in the occupational environment. This has led to concern regarding its use as a petrol additive, given the comparison with the use of lead in petrol and its associated problems. The health aspects of Mn intake have been considered in previous publications by U.S. EPA (1984), Canadian Health and Welfare (1978) and Cooper (1984). In this paper, the debate regarding the use of MMT is continued and the public health issues of increased atmospheric Mn_3O_4 levels discussed.

EXPOSURE LEVELS

Exposure of humans to MMT, apart from occupational exposure during production, is limited to the inhalation of MMT in urban air or inhalation of vapours of petrol/MMT mix at petrol stations. MMT undergoes rapid decom-

Note: The views expressed in this paper do not necessarily reflect the views of the Australian National Health and Medical Research Council.

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position in sunlight to a mixture of manganese oxides. The half-life for the photolysis of MMT in air and sunlight has been estimated to be ~15 s (Ter Harr et al., 1975). MMT levels were measured in Toronto by Coe et al. (1980) and could be detected only in an underground car park at levels of 0.1–0.3 ng m^{-3}. No MMT could be detected in urban air above the detection limit of 0.05 ng m^{-3}.

Exposure to MMT in petrol has been compared to exposure to tetraethyllead (TEL) in petrol (Faggan, 1975). With TEL at 2.5 g Pb gal^{-1} and MMT at 0.125 g Mn gal^{-1}, the partial pressure of MMT in petrol was calculated to be about one-thirtieth that of TEL. Thus, at these levels in petrol, exposure to MMT would be much less than exposure to TEL. While the inhalation toxicity (measured as LC_{50} in rats) for MMT (100 mg m^{-3}) is greater than for TEL (850 mg m^{-3}), the overall hazard should be less.

The use of MMT would be expected to result in increased atmospheric levels of Mn_3O_4, the principal exhaust product. Traces of manganic oxide (Mn_2O_3) may also be present. In the U.S., data from the National Air Surveillance Network estimated the current average urban atmospheric Mn concentration to be 0.03 $\mu g\ m^{-3}$ (U.S. EPA, 1984). A prediction of the likely increase in the average Mn concentration in urban air from the use of MMT can be made by using the ratio of the current lead levels in petrol and air. While not altogether accurate, since the physical properties of the lead and Mn particulates could be different, the figure obtained is probably a reasonable estimate. In Australia, where lead is currently added to petrol at a level of 0.45 g l^{-1}, an average airborne lead concentration of 0.5 $\mu g\ m^{-3}$ was found in Sydney (Roberts et al., 1983). It is clear from the range of values obtained in a number of Australian cities that airborne lead levels can be 10 times this value or higher in areas of high traffic density. Based on the average airborne lead value of 0.5 $\mu g\ m^{-3}$, and a Mn level in petrol of 17 mg l^{-1}, the expected increase in airborne Mn concentration would be 0.02 $\mu g\ m^{-3}$. In areas of high traffic density, therefore, values of 0.2 $\mu g\ m^{-3}$ could be expected. While a level of 0.2 $\mu g\ m^{-3}$ would be a significant increase in Mn levels from the current ambient urban levels of 0.01 $\mu g\ m^{-3}$ found in Australian cities (Goodman, 1976; Roberts et al., 1983), the levels are still low compared with airborne lead levels in urban areas.

MANGANESE AND HUMAN HEALTH

Manganese is the 12th most abundant element and exhibits 11 oxidation states. It has been shown to be essential for growth and reproduction in a number of species, including man, and although the daily requirement is unknown, a diet containing 50 ppm is considered adequate for laboratory animals. The major industrial use for manganese, both alloys and metal, is in steel production. It is also used in the chemical industry and in battery production. The major sources of Mn in the atmosphere are ferroalloy manufacture, iron and steel manufacture and fossil fuel combustion.

Human exposure to manganese can occur by inhalation or by ingestion. Absorption via the lung is highly dependent on particle size, and those larger

than 15 μm are unlikely to reach the thoracic (tracheobronchial and alveolar) region. Particles of 10 μm do not reach the alveoli, but ~35% are deposited tracheobronchially. Alveolar deposition is greatest for particles in the 2–4 μm range and although nearly all particles smaller than 2 μm reach the alveoli, many remain suspended and are exhaled. All particles < 2 μm can be conservatively considered to be deposited in the alveoli (U.S. EPA, 1984). Moore et al. (1975) has estimated the size of Mn_3O_4 particles from combustion of petrol containing MMT to be ~0.26 μm and therefore capable of alveolar deposition. While the rate of lung absorption of inhaled Mn in both animals and humans is unknown, the U.S. Task Group on Metal Accumulation (1973) considered only particles less than a few tenths of a micrometre in diameter to be eventually absorbed into the blood. If all the inhaled Mn_3O_4 were absorbed, even at a point of high traffic density (0.5 $\mu g\,m^{-3}$), the maximum Mn intake would be 10 μg (assuming an adult breathes 20 $m^3\,day^{-1}$). The actual intake would be less than this given than an unknown fraction of the inhaled particles would be exhaled immediately and another unknown fraction would be removed by mucociliary clearance.

The possibility that Mn oxides may be absorbed differently in the lung to other Mn compounds may have been answered partially by the experiments of Mena et al. (1969). In these experiments, an airborne aqueous solution of either 54-$MnCl_2$ or 54-MnO_2 was absorbed (either in the lung, GI tract or both) after inhalation exposure. Excretion rates in the urine were similar for both compounds and the results suggest that 60–70% of the inhaled Mn was eventually swallowed and absorbed via the GI tract.

With regard to oral intake, WHO (1973) has estimated that the daily dietary intake of Mn ranges between 2 and 3 $mg\,day^{-1}$ for adults. Intake for infants is much lower (0.002–0.004 $mg\,kg^{-1}\,day^{-1}$) due to the low concentration of Mn in both breast and cow's milk. U.S. EPA (1984) data on the concentration in drinking water indicates that the level is very low (median level 4 $\mu g\,l^{-1}$) and not a significant source of dietary Mn. Gastrointestinal absorption of Mn in adults is likely to be less than 5% of the total Mn ingested (WHO, 1981). In anaemic subjects, the rate is probably higher, given that the transport mechanism for Mn and Fe are the same (Mena et al., 1974). Clearance from the respiratory tract is an even smaller source of Mn. The U.S. EPA (1984) estimates an average ingestion of 0.00026 $mg\,day^{-1}$ by this route, assuming 100% deposition and clearance at an ambient exposure level of 0.023 $\mu g\,m^{-3}$.

COMPARISONS WITH LEAD

A number of the toxicological concerns regarding increased airborne levels of Mn_3O_4 from the use of MMT appear to have arisen by comparison with the known toxic effects of lead. A major reason why this is an inappropriate analogy is that Mn is already present in the diet and is absorbed via the GI tract at a high level in comparison to the expected level of pulmonary absorption of the Mn oxides. While Mn may be present in a variety of salts and oxides in food

and air, and the relative absorption rates of these forms of Mn may vary, their toxicological effects are considered to be identical following absorption.

Lead, on the other hand, is not a normal or useful component of the diet and its toxic effects are well characterized at low levels of exposure. While blood lead levels are a reasonably good indicator of recent lead exposure, the levels of Mn in blood or urine are an extremely unreliable indicator of recent or long-term exposure due to rapid removal of Mn from the blood stream ($t_{½}$ = 1.5 min). Levels of Mn in blood do not parallel the presence of psychological or neurological symptoms, and display wide individual variation (Roels et al., 1987b). The Mn level in human tissues also has a relatively short half-life (liver, $t_{½}$ = 25 days). No useful comparison, therefore, can be made between blood Mn and Pb levels.

As discussed earlier, comparisons with lead have been useful for estimating expected airborne levels of Mn resulting from the use of MMT. A further comparison might also be useful for determining the likely route of intake of Mn following exposure to Mn_3O_4. Lead-containing particles from car exhausts are absorbed by inhalation or by the ingestion of dust particles on food or other objects in the environment. For children, in particular, the ingestion route is the most significant and the inhalation route is considered a minor exposure pathway. Hence the high blood lead levels found in children living near areas of high traffic density (U.S. DHHS, 1985). Since there is already a high dietary intake of Mn in both children and adults, the contribution from Mn_3O_4-containing dust will not significantly increase Mn intake.

PULMONARY TOXICITY

Inhaled metallic particles can be considered to have one of three fates (Adkins et al., 1980): (i) removal from the lung by exhaled air, by mucociliary mechanisms, by engulfment by pulmonary macrophages, or by lymphatic clearance; (ii) deposited in the lung tissue over a long period with little or no harm; (iii) passage into the systemic circulation. The fate of a particular particle will depend to a large extent on its size, and inhaled Mn particles are expected to be cleared by several of the above mechanisms.

Occupational exposure to Mn dust leading to a high rate of pneumonia has been studied in a large number of epidemiological surveys (see U.S. EPA, 1984). Exposure to high levels of Mn is associated with a syndrome known as 'manganese pneumonia'. The levels required to observe symptoms are generally $> 5\,mg\,m^{-3}$, which is the present limit in the United States for occupational exposure. However, exposure/response relationships are limited by the variable exposure conditions and the number of measurable end points. One study (Nogawa et al., 1973), conducted with Japanese schoolchildren whose school was close to a ferromanganese plant, found there was an increased prevalence of respiratory symptoms (e.g. sputum, wheezing and sore throat) at particularly low exposure levels (3–11 $\mu g\,m^{-3}$). No other study has confirmed these results at such low exposure levels.

Extensive studies in animals suggest that Mn is capable of producing a primary inflammatory reaction in the lung. This condition appears to be exacerbated by secondary bacterial infection leading to bronchopneumonia or pneumonitis and chronic inflammatory effects such as fibrosis. Several studies indicate that exposure to Mn has a depressive effect on the number and phagocytic capacity of alveolar macrophages (see U.S. EPA, 1984). Thus the observed lung changes may be attributed to decreased resistance to respiratory infection.

The majority of studies have been conducted with MnO_2 or $MnCl_2$. Chronic inhalation studies in rats and monkeys using Mn_3O_4 were conducted by Ulrich et al. (1979a,b,c). No significant changes were reported in any of the biochemical or pathological parameters measured, even at the highest dose level ($1152\,\mu g\ Mn\,m^{-3}$). The objective tests of pulmonary function (mechanical and ventilatory properties), however, were not extensive enough to draw any firm conclusions. The possibility of synergistic effects on pneumonitis development as a result of bacterial or viral infection was not assessed. In a similar study in monkeys by Coulston and Griffin (1976), exposure to Mn_3O_4 at a dose level of $100\,\mu g\,m^{-3}$ produced no gross pathological changes and no histopathological changes in the lung. This Mn exposure level was 2–3 orders of magnitude higher than that expected from the use of MMT in petrol.

In an epidemiological study of male workers in a Mn oxide and salt producing plant (Roels et al., 1987a), respiratory tract problems were assessed by a questionnaire as well as by measurement of ventilatory performance. For non-smokers, the frequency of cough and sputum in the cold season and also recent episodes of bronchitis were increased but not to the level of statistical significance. For smokers, the frequency of cough was significantly different to controls. Acute bronchitis was also more prevalent in Mn workers than controls. The difference between Mn workers and controls for acute bronchitis over the last 3 years was particularly striking (38% compared with 19%).

Ventilatory performance of Mn exposed non-smokers was only slightly but significantly different to the performance of controls. No additional changes were observed in Mn exposed smokers. The authors conclude that mild respiratory signs and symptoms may occur in workers exposed to an average airborne Mn concentration of $1\,mg\,m^{-3}$ over a number of years.

NEUROTOXICITY

The effects of manganese on the CNS in both humans and animals is quite well documented and has been reviewed recently by the U.S. EPA (1984). A brief synopsis is given here, together with a consideration of two recent papers.

Advanced manganese poisoning is described in a syndrome known as 'manganism'. The CNS toxicity can be divided into two stages, the first of which is characterized by psychological disturbances and may be reversible if Mn exposure is terminated. The second stage is a neurological disturbance which is not reversible. The disease begins with anorexia, asthenia and occasional

psychotic behaviour. This can be followed by slurred speech, mask-like face, clumsiness, indifference, spasmodic laughter and crying spells. Severe symptoms are limb rigidity, tremors, excessive salivation and sweating. The symptoms resemble Parkinson's syndrome.

Cases of manganism have been identified in all of those industries associated with the production and use of Mn which produce a high concentration of Mn dusts and fumes. A large number of cases have occurred in Mn mines. Manganese poisoning can result from occupational exposure to Mn dust after only a few months of exposure. The frequency of reporting of the disease is low at exposure levels $< 5\,mg\,m^{-3}$. A few signs of exposure (e.g. tremors at rest) have been reported at exposure levels as low as $0.3\,mg\,m^{-3}$, but this level is still 3–4 orders of magnitude higher than that likely to arise from the use of MMT in petrol.

While a large number of studies on the effects of Mn on the CNS in animals is available, the use of different routes of administration, as well as the measurement of different end points (behavioural, biochemical, histological) has not enabled a clear dose–response relationship to be established. The appropriateness of some animal species for studying Mn toxicity has also been questioned. Rats do not exhibit the wide range of behavioural manifestations described in primates and may not accurately model the neurological disorders observed in man. Exposure of monkeys to $1152\,\mu g\,m^{-3}$ for $24\,h\,day^{-1}$ for 9 months produced no evidence of Mn toxicity (Ulrich et al., 1979a,b,c).

Animal experiments have helped to determine the mechanism of Mn toxicity. Evidence that disturbance of brain neurotransmitter metabolism represents a key effect is accumulating. Chronic exposure leads to a decrease in brain monoamine levels, particularly dopamine (Gianutsos and Murray, 1982). The measurement of brain Mn concentrations following chronic exposure may lead to a better understanding of dose–response relationships in animals. A recent paper by Bird et al. (1984) on the Mn level in monkey brain during a 2-year exposure has addressed this problem. After exposure to levels of $30\,\mu g$ $MnO_2\,m^{-3}$, dopamine levels in both caudate and globus pallidus were depressed while Mn levels in the globus pallidus were increased. No neurological changes were observed during the exposure period and the results suggest that exposure at levels $> 30\,\mu g\,m^{-3}$ or over longer periods may be required to observe such changes. Since, in humans, the first signs of toxicity appear as psychiatric manifestations, this may indicate the involvement of dopamine pathways which project to the frontal lobe. This study strongly suggests that neurological changes cannot be expected in humans until high levels of exposure are reached.

In an epidemiological study of Mn workers in a Mn oxide and salt producing plant by Roels et al. (1987a), subjective symptoms, psychomotor tests, and neurological examinations were recorded. Significant increases in the level of fatigue, timitis, trembling of fingers and irritability were noted. Neurological examination revealed differences in trunk rigidity only. Psychomotor tests revealed alterations in simple reaction time, audioverbal short-term memory

capacity and hand tremor. The authors estimate that a time-weighted average exposure to airborne Mn dust of $\sim 1\,mg\,m^{-3}$ over a number of years may lead to the occurrence of pre-clinical signs of intoxication.

OTHER CHRONIC TOXICITY

Two other possible areas of Mn toxicity have been considered, namely oncogenicity and reproductive effects. Given the relatively low intake of Mn into the bloodstream after airborne Mn_3O_4 exposure, an oncogenicity study by the inhalation route could only be useful for the detection of lung tumours. Since there has been no reported increased incidence of lung tumours among occupationally exposed individuals, often at very high doses (see WHO, 1981), the likelihood of Mn being a lung carcinogen seems small. In a paper by Stenback and Rowland (1979), intratracheal instillation of MnO_2 dust (1.5 mg once a week for 20 weeks) into hamsters did not increase the incidence of lung tumours and nor did it enhance the level of tumours produced by concurrent instillation of benzo[a]pyrene.

Interest in the possible effects of Mn on reproductive parameters in males has centred around reports by manganese workers of impaired sexual behaviour in the form of diminished libido and impotence. Animal experiments have thus concentrated on morphological and biochemical changes in testes. In the experiments of Chandra et al. (1971, 1973) degenerative changes in semeniferous tubules were observed after 150 days of intraperitoneal administration of $MnCl_2$ at a dose of $8\,mg\,kg^{-1}\,day^{-1}$. Other studies have not been in agreement with the Chandra results, and no definite effects of Mn after oral or inhalation exposure can be identified. The only animal study performed with Mn_3O_4 is that by Laskey et al. (1982). Serum testosterone levels and epididymal sperm counts were depressed at dose levels $> 35\,mg\,kg^{-1}$, which are well above the likely human exposure dose level.

In an epidemiological study by Lauwerys et al. (1985), the number of children fathered by workers exposed to a median Mn concentration of $0.97\,mg\,m^{-3}$ was significantly lower than the expected number. This level of exposure is approximately 3–4 orders of magnitude higher than the Mn exposure level likely to arise from the use of MMT.

CONCLUSIONS

Potential toxicological effects of increased airborne Mn appear to be restricted to the pulmonary and central nervous systems. Effects in the lung such as inflammation, pneumonia and bronchitis are clearly evident in both animals and humans exposed to relatively high levels of Mn, however individual susceptibility and toxic manifestations seems to vary greatly, even after very long exposure periods. Further low level chronic exposure experiments in animals may be necessary to define a no-effect level, particularly with regard to ventilatory performance, which appears to be one of the most sensitive indicators

of lung damage. Further studies are also necessary to measure possible changes in susceptibility to respiratory infection after low-level chronic exposure. The data available at present, however, indicate that the increased level of airborne Mn in the form of Mn_3O_4 generated by the combustion of MMT would be approximately three orders of magnitude lower than the level necessary to produce adverse effects in the lung.

Similarly with regard to CNS effects, both animal and human studies to date suggest that relatively high levels of Mn intake are required to produce symptoms of toxicity. In humans, the long time periods required to produce symptoms suggest a cumulative effect. Further research into the mechanism of Mn-initiated CNS effects may lead to a better understanding of dose–response relationships.

On the basis of present information, there is no toxicological evidence to suggest that the increased level of airborne Mn resulting from the combustion of MMT as a petrol additive is likely to constitute a health risk to the general population.

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Origins of Manganese in Air Particulates in California

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The proportions of manganese to other metals in samples of airborne fine particles taken at some sites in California have increased greatly since the beginning of 1985. Here, data are presented which indicate that the addition of manganese to leaded gasoline is largely responsible for this increase. Concentrations of manganese, silicon, titanium, iron, lead and other elements in airborne particles were measured using energy-dispersive X-ray fluorescence analysis. Coefficients of correlation among levels of manganese, iron and lead measured at twenty sites in California were calculated. Levels of manganese and iron are generally highly correlated because of the presence of large amounts of these elements in the earth's crust. Levels of airborne manganese and lead at sites in Southern California are often highly correlated, suggesting a vehicular source of manganese. Observed manganese concentrations are apportioned into two major sources: the earth's crust and motor vehicles. The apportionment indicates that vehicular emissions of manganese may account for a significant part of the total at urban sites in Southern California. At most other sites, the contribution from vehicles is found to be far smaller than that from the earth's crust.

The California Air Resources Board (CARB) currently measures levels of manganese in airborne particles as part of its toxic air contaminants program. Levels of manganese in California range from less than 10 to more than 200 nanograms per cubic meter. Manganese along with many other elements is measured by energy-dispersive x-ray fluorescence spectrometry (EDXRF). EDXRF measurements are also performed on samples of fine and coarse particles collected at several locations using dichotomous samplers. Recently, it was observed that the proportions of manganese to other elements of largely crustal origin such as titanium, iron and silicon were greatly increased in samples of fine particles, but not in coarse particles. Later, it was found that manganese is added to leaded gasoline as a partial substitute for lead. These observations suggested that motor vehicles could be a significant source of manganese. Therefore, it was of interest to attempt to apportion these measurements of total manganese into contributions from its major sources.

Sampling and Analysis

Samples of total airborne particles at twenty sites in California were collected using low-volume samplers. Samples of fine and coarse particles at five sites using dichotomous samplers were also collected. These samplers had a particle-size cutoff of about ten microns. The sampling period was 24 hours and the sampling rate was about one cubic meter per hour for both types of samplers. Teflon filters were used to collect the samples. The samples were analyzed using EDXRF. The limits of detection (LOD) for manganese, iron, and titanium are about 1 ng/m^3. The LOD for lead is about 3 ng/m^3, and about 10ng/m^3 for silicon. Most of the measurements of these elements were well above their limits of detection, except for some values of manganese in fine-particle samples taken at sparsely populated locations.

Major Sources of Manganese

Here, sources of manganese in California are considered to be in three major categories: the earth's crust, industry and vehicular fuel combustion. The earth's crust is undoubtedly an important source because manganese constitutes, on average, 953 ppm of it.[1] In the past, measurements of airborne manganese and other elements abundant in the earth's crust were often in roughly the same proportions as their crustal abundances.[2,3] Also, dust from construction and roads is considered to be in this category.[3]

Industry is expected to make small contributions to levels of airborne manganese at most sites in California. Almost all of the industrial sources of manganese in the USA are located east of the Mississippi river, according to a recent EPA report.[4] It stated that in California there are no ferromanganese plants, no production of manganese metal or oxides of manganese, no blast furnaces, no open hearth furnaces, no oxygen furnaces, and no sinter plants. Southern California does have eight cement plants, while Northern California has four. Electric arc furnaces are located in Los Angeles and in Carson, California.[4] Emissions from these plants are controlled, but the output of manganese is not known. According to an emissions inventory by Cass and McRae[3] for the Southern California air basin in 1976, only seven percent of the airborne manganese was emitted from noncrustal sources.

Combustion of leaded gasoline is now expected to contribute to levels of airborne manganese. Major oil refiners began adding an anti-knock compound containing manganese to leaded gasoline in 1985 or late 1984 to compensate for the reduced lead levels required by both CARB and EPA. As mentioned above, elevated proportions of manganese have been observed by the CARB in fine particles collected at four out of five locations in California since 1985. The exceptional location was at China Lake, a remote area in the Mojave desert. The ratio of manganese to other elements of a largely crustal origin in fine particles is generally greatest at the three Southern California sampler locations, which are in Long Beach, in the Riverside-Rubidoux area, and in the Azusa-Glendora area. The ratio of manganese to silicon reached 0.03 to 0.05 at these three sites, whereas at China Lake, it never exceeded the average crustal ratio of 0.0036[1] by more than 20 percent. These observations are consistent with a widespread anthropogenic source of manganese such as combustion of gasoline.

In Figure 1 is shown the trend in ⟨Mn⟩/⟨Si⟩ (as filled circles) for the dichotomous sampler located in the River-

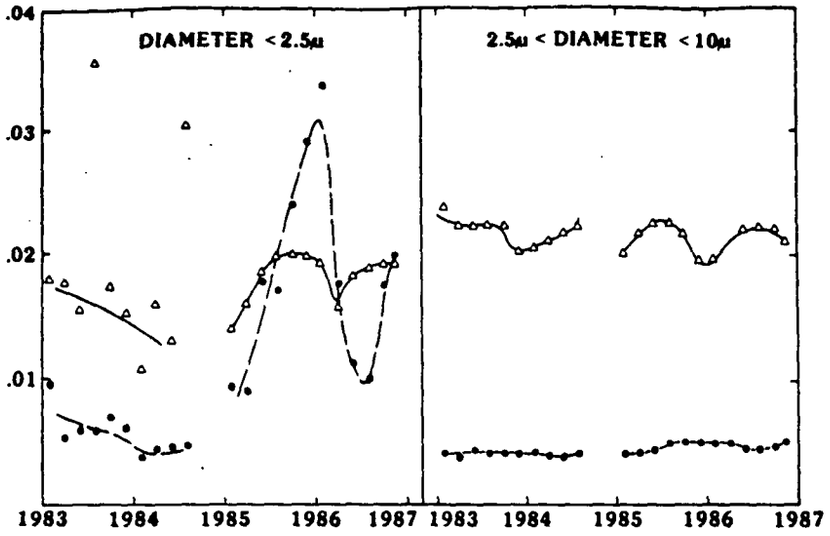

Figure 1. Ratios of two-month average levels of manganese (circles) and titanium (triangles) to those of silicon for the years of 1983 through the third quarter of 1986 at Riverside-Rubidoux.

side-Rubidoux area, about forty miles east of Los Angeles. This is classified as a receptor site because many of the pollutants found in this area are generated in the large metropolitan area to the west of it. The fine-particle ratio ⟨Mn⟩/⟨Si⟩ increased from about 0.005 in 1984 to over 0.03 in early 1986. The large difference in the trends of the fine- and coarse-particle ratios suggests a recent additional source of fine-particle manganese. The ratios of average values of titanium, another "crustal" element, to those of silicon, are plotted in Figure 1 (triangles) for comparison. These ratios change far less than does the fine-particle ⟨Mn⟩/⟨Si⟩ ratio. They are generally close to the average crustal ratio of 0.016.[1] There seem to be seasonal variations in both the fine and coarse ratios of titanium to silicon and possibly in the fine-particle ratio of manganese to silicon. The ratios of iron to silicon were also checked and found to vary about the same as did the ⟨Ti⟩/⟨Si⟩ ratios.

In Figure 2 are shown the same ratios for dichotomous samples taken in Long Beach, which is considered a pollutant-source location. The trends in the ratios are similar to those at Riverside-Rubidoux. However, the fine and coarse ⟨Ti⟩/⟨Si⟩ ratios at Long Beach are generally higher than the corresponding crustal ratio. The points plotted in Figures 1 and 2 are ratios of bimonthly averages of XRF data from dichotomous samples taken every six days.

Further, data were obtained on the amount of manganese added to leaded gasoline by major oil refiners in California in recent years. These are ARCO, Chevron, Exxon, Mobil, Shell, Texaco, and Union 76. Only ARCO and Texaco added manganese to leaded gasoline between 1984 and 1986. Quarterly totals of manganese added (tons) by these refiners[5,6] for 1984 through the fourth quarter of 1986 are shown in Figure 3. Since 1984, addition of manganese to leaded gasoline has increased greatly. Comparison of Figures 1 and 2 with Figure 3 shows roughly similar trends in the fine-particle ratio ⟨Mn⟩/⟨Si⟩ and in manganese added to leaded gasoline. In these Figures there is a large increase starting in late 1984 or early 1985, a maximum early in 1986 and another increase during the fourth quarter of 1986. However, the correspondence is not exact. The graph of added manganese shows a

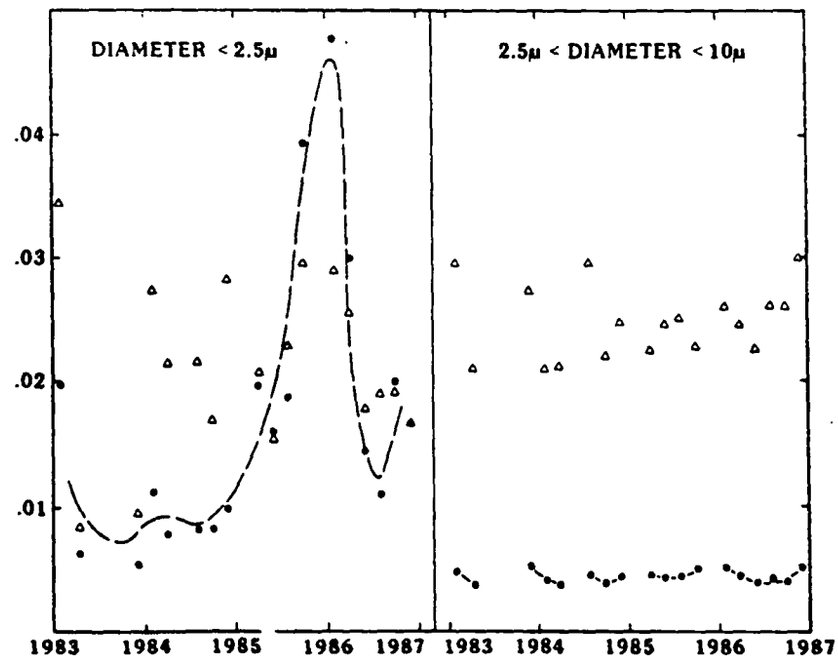

Figure 2. Ratios of two-month average levels of manganese (circles) and titanium (triangles) to those of silicon for the years of 1983 through the third quarter of 1986 at Long Beach.

decrease in the fourth quarter of 1985 which is not repeated in the fine-particle ⟨Mn⟩/⟨Si⟩ ratios at either site. More importantly, this ratio changes much more throughout 1986 than does the added manganese.

These differences indicate that this ratio is affected by factors other than addition of manganese to gasoline, possibly those related to changes in season. Before 1985, there were no definite seasonal variations observed in the fine-particle ⟨Mn⟩/⟨Si⟩ ratio at the sites in Riverside-Rubidoux and Long Beach. However, seasonal variations were observed consistently for several years in the ratio of lead to

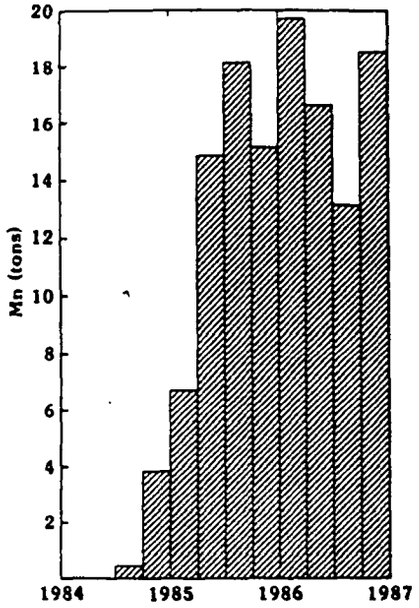

Figure 3. Quarterly totals of manganese (tons) added to leaded gasoline sold in California by major oil refiners.

silicon in fine particles from these sites. These are shown in Figure 4. Maxima in this ratio are generally observed between November and February. The general decrease in the lead maxima with time is probably due for the most part to the gradual decrease in lead emissions from motor vehicles. At these sites both the ⟨Pb⟩/⟨Si⟩ and ⟨Mn⟩/⟨Si⟩ ratios in fine particles change similarly between the middle of 1985 and the end of 1986, but the ⟨Mn⟩/⟨Si⟩ ratio changes considerably more.

At both sites, the ⟨Mn⟩/⟨Si⟩ peak in late 1985 is much higher than that observed in 1986. It is possible that differences in seasonal factors such as precipitation and surface inversion levels affect the ⟨Mn⟩/⟨Si⟩ ratio in fine particles. Higher levels of carbon monoxide and oxides of nitrogen in the last months of 1985 in Southern California have been attributed to generally lower surface inversion levels.[7]

Source Apportionment

The linear apportionment model used here for manganese has been applied before by other workers for multielement apportionment.[3,8] In this paper, it is assumed that the observed total manganese is the sum of contributions from motor vehicles, the earth's crust, and from other unnamed sources, S:

$$(Mn)_{total} = (Mn)_{veh} + (Mn)_{crust} + S$$

Next, it is assumed that both $(Mn)_{veh}$ and (Pb) are proportional to the total emissions E_{veh} from vehicles:

$$(Mn)_{veh} = cE_{veh}$$
$$(Pb) = c'E_{veh}$$

Therefore, $(Mn)_{veh}$ equals $k_{veh}(Pb)$, where k_{veh} is c/c'. The factors c and c' are not strictly constant due to changes in parameters such as concentrations of lead and manganese in gasoline. But as long as c and c' change by about the same degree, k_{veh} will be approximately constant. Similarly, $(Mn)_{crust}$ becomes $(Mn)_{crust} = k_{crust}(Fe)$ where Fe is a tracer element for the earth's crust analogous to the use of Pb for vehicular emissions. We chose iron instead of silicon as a crustal tracer element because iron is more accurately measured by XRF, especially when the samples are heavily loaded. Corrections for x-ray attenuation by sample thickness and particle size are much larger for silicon than for iron.[9]

The k values were obtained as usual by a linear least-squares fit of the observed total manganese concentrations to the observed lead and iron concentrations. The contribution S from unnamed sources was assumed to be constant and obtained as the intercept of the linear regression. The average vehicular fraction of the total manganese was defined as k_{veh} ⟨Pb⟩/⟨Mn⟩ and the average crustal fraction as k_{crust}⟨Fe⟩/⟨Mn⟩. It is more proper to use ⟨Pb/Mn⟩ and ⟨Fe/Mn⟩, but the ratio of the averages is less sensitive to experimental errors in the elemental concentrations.

Samples from all twenty sites of low-vol samplers were available from the middle of 1985. Two six-month time periods were chosen: July to December of 1985 and January to

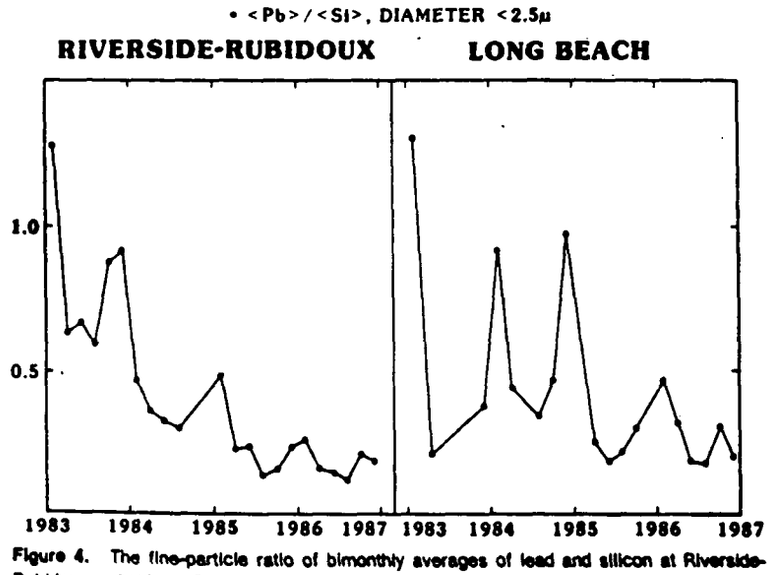

Figure 4. The fine-particle ratio of bimonthly averages of lead and silicon at Riverside-Rubidoux and at Long Beach.

Table I. Source apportionment.

Site	Sampling period	⟨Mn⟩ (ng/m³)	Vehicular fraction	Crustal fraction	r^2 Pb-Mn	r^2 Fe-Mn	r^2 Pb-Fe	Multiple r^2
Central Valleys								
Bakersfield	7–12, 1985	43	−0.02	1.00	0.02	0.99	0.02	0.99
	1–6, 1986	36	0.01	1.06	0.03	0.98	0.04	0.98
Citrus Hts.	7–12, 1985	24	0.17	0.95	0.22	0.95	0.11	0.98
	1–6, 1986	16	0.18	0.89	0.13	0.87	0.03	0.91
Fresno	10–12, 1985	23	0.18	0.96	0.72	0.99	0.66	0.99
	1–6, 1986	24	0.02	0.96	0.0	0.99	0.0	0.99
Merced	7–12, 1985	27	0.13	0.93	0.19	0.98	0.11	1.00
	1–6, 1986	18	0.02	0.99	0.09	0.99	0.08	0.99
Modesto	7–12, 1985	55	0.24	0.81	0.65	0.96	0.53	0.98
	1–6, 1986	15	0.0	0.93	0.01	0.98	0.01	0.98
Stockton	1–6, 1986	53	0.11	0.93	0.75	0.97	0.71	0.98
Average		30	0.09	0.95	0.26	0.97	0.21	0.99
Bay Area								
Concord	7–12, 1985	8	0.09	1.00	0.45	0.98	0.41	0.98
	1–6, 1986	9	−0.04	1.02	0.43	0.96	0.48	0.97
Fremont	7–12, 1985	14	0.17	0.85	0.53	0.96	0.43	0.98
	1–6, 1986	11	0.17	0.92	0.62	0.97	0.53	0.98
Richmond	7–12, 1985	11	0.31	0.93	0.34	0.89	0.19	0.92
	1–6, 1986	11	0.16	0.88	0.55	0.89	0.50	0.90
San Francisco	7–12, 1985	13	0.76	0.36	0.74	0.69	0.68	0.78
	1–6, 1986	12	−0.06	0.99	0.68	0.95	0.74	0.95
San Jose	7–12, 1985	36	0.17	0.89	0.51	0.94	0.35	0.97
	1–6, 1986	29	0.16	0.89	0.70	0.97	0.56	0.99
Average		15	0.19	0.87	0.56	0.92	0.49	0.94
Southern California								
Chula Vista	7–12, 1985	15	0.69	0.54	0.77	0.72	0.43	0.90
	1–6, 1986	13	0.16	0.90	0.11	0.79	0.01	0.86
El Cajon	7–12, 1985	27	0.65	0.50	0.73	0.86	0.39	0.98
	1–6, 1986	23	0.52	0.65	0.73	0.78	0.37	0.94
El Monte	7–12, 1985	38	0.49	0.70	0.82	0.86	0.69	0.92
	1–6, 1986	37	0.66	0.44	0.87	0.83	0.63	0.95
Long Beach	7–12, 1985	42	0.41	0.63	0.57	0.77	0.22	0.93
	1–6, 1986	30	0.59	0.45	0.91	0.84	0.62	0.98
Los Angeles	7–12, 1985	35	0.01	1.21	0.54	0.90	0.59	0.90
	1–6, 1986	37	0.75	0.37	0.45	0.88	0.81	0.96
Rubidoux	7–12, 1985	45	0.26	0.77	0.84	0.95	0.78	0.96
	1–6, 1986	58	0.36	0.76	0.63	0.95	0.46	0.98
Santa Barbara	7–12, 1985	12	0.55	0.48	0.85	0.68	0.35	0.97
	1–6, 1986	13	0.62	0.24	0.82	0.51	0.18	0.96
Simi Valley	7–12, 1985	28	0.38	0.63	0.67	0.88	0.38	0.97
	1–6, 1986	19	0.65	0.35	0.35	0.77	0.05	0.93
Upland	7–12, 1985	33	0.18	0.71	0.51	0.96	0.42	0.97
	1–6, 1986	32	0.32	0.64	0.22	0.90	0.05	0.98
Average		30	0.43	0.64	0.68	0.84	0.45	0.95

June of 1986. Samples were taken every six days at sites in Northern California and every twelve days at the Southern California sites. Therfore, about fifteen data sets were used in the linear regressions for the sites in Southern California and twice that many for the Northern California sites.

The multiple correlation coefficients (r^2) of the linear fits are generally above 0.9, as are the correlation coefficients between iron and manganese [r^2(Fe-Mn)]. It may be inferred from this alone that the crustal contribution to airborne manganese is very important in California. However, at many of the sites in Southern California, r^2(Pb-Mn) is comparable to r^2(Fe-Mn). It is for these sites that source apportionment is most useful. Table I gives values of r^2, source fractions, and average levels of manganese at each of the sites.

The sampler sites have been segregated into three regions in Table I: Southern California, including San Diego; the San Francisco Bay area; and the San Joaquin Valley plus the Sacramento Valley. For the sites within these regions, the various correlation coefficients and source fractions tend to be closer to each other than to those of sites in other regions. The multiple correlation coefficients (r^2) of the linear regressions are highest on average for the locations in the San Joaquin-Sacramento Valley. All except one were equal to or greater than 0.98 for the sampling periods in this region. Values of r^2(Fe-Mn) were also the highest, whereas values of r^2(Pb-Mn) were the lowest of the three regions on the average. The source apportionment model gave an average crustal fraction of 0.95 and an average vehicular fraction of only 0.09 for sites in this region.

The results for the locations in the Bay area fall between those of other regions, but they are closer to those for the San Joaquin-Sacramento Valley. The average multiple r^2 is 0.94 for this region. Again, the average crustal fraction (0.87) far exceeds the average vehicular fraction (0.18). The results for San Francisco are very inconsistent and therefore doubtful.

The sites in Southern California are calculated to have the highest average vehicular fraction, 0.43, compared to an average crustal fraction of 0.64. The results for Los Angeles are inconsistent, as they were for San Francisco, suggesting that our two-source model is inadequate for such large urban centers. However, as mentioned earlier, previous work[3] found no evidence for other major sources of manganese in the Los Angeles area. The results for Long Beach, a smaller urban area, are more consistent. They suggest a substantial vehicular contribution. Six of the Southern California locations may be paired according to their mutual proximity and to the similarity of the results of the two-source model.

Table II. Dichotomous results.[a]

Site	Sample Period	⟨Mn⟩ fine	$\langle Mn\rangle_{veh}$ fine	⟨Mn⟩ coarse	$\langle Mn\rangle_{crust}$ coarse	$\langle Mn\rangle_{veh}$ fine plus coarse	$\langle Mn\rangle_{crust}$ fine plus coarse
Long Beach	1/86–6/86	11	7	9	7	8 (18)	12 (14)
Riverside	7/85–12/85	15	9	28	27	9 (12)	34 (35)
Rubidoux	1/86–6/86	10	10	21	19	12 (21)	19 (44)

[a] Mn concentration in ng/m³. Results for total low-vol particles in parentheses.

These are Chula Vista-El Cajon, which are in the San Diego area; Santa Barbara-Simi Valley, which are north and west of the primary L.A. metropolitan area; and Upland-Riverside-Rubidoux, which are directly east of Los Angeles. Our model predicts high vehicular fractions at the Chula Vista-El Cajon and Santa Barbara-Simi Valley sites. The magnitudes of the vehicular contributions are not large, however, because the total manganese levels are relatively small at these four sites. The calculated average vehicular contribution of Mn at all Southern California sites was about 13 ng/m³, which was around four times the value calculated for the other two regions.

The source apportionment model was also applied to measurements on dichotomous samples from two sites in Southern California: Long Beach and Riverside-Rubidoux. The results are shown in Table II. The model predicted that at these sites, on the average, most of the fine-particle manganese came from motor vehicles, and almost all of the coarse-particle manganese came from the earth's crust. The vehicular contribution is predicted to be 20 to 40 percent of the total manganese.

For comparison of the total particulate and dichotomous results, we put the source contributions for the samples of total particles in parentheses in Table II next to the dichotomous fractions. The agreement between the two results is only fair. The crustal contributions to total particles were expected to be greater because the dichotomous sampler does not collect particles of diameter greater than 10 μm, most of which should come from the earth's crust. But the differences in $\langle Mn\rangle_{veh}$ for total particles exceed the $\langle Mn\rangle_{fine}$. Assuming that most of the vehicular contribution appears in the fine fraction, this suggests that the calculated $\langle Mn\rangle_{veh}$ (total particulates) for these sites are somewhat high.

The factors k_{veh}, k_{crust}, and S will be discussed below. They all have physical interpretations which make them of interest and their values should be physically reasonable if the model is valid. First, k_{crust} is obviously the ratio $(Mn)_{crust}/(Fe)$. Therefore, our values of k_{crust} should be close to the average crustal ratio of these elements, 0.017,[1] barring differential rates of suspension or fallout between Mn and Fe. Our calculated average k_{crust} for the total particles is not far from this, at 0.015, which tends to support the results of our model. The value of k_{crust} decreases from 0.0169 for the San Joaquin-Sacramento Valley region to 0.0147 for the Bay area sites, and to 0.0136 for Southern California sites. This variation is not understood at present.

The factor k_{veh} is the ratio $(Mn)_{veh}/(Pb)$. Therefore, it should be related to the ratios of the quantities of manganese and lead which are emitted from motor vehicles, and perhaps to this ratio in leaded gasoline. The model gives the following averages of k_{veh} for the three large regional divisions of the sampling sites: 0.10 for Southern California and 0.03 for the other two major regions.

For the following reasons it is expected that levels of manganese in leaded gasoline will be higher in Southern California, and therefore that k_{veh} will be higher for sites located there. Because it is hazardous to transport gasoline over large distances, oil companies generally buy or exchange from other refiners the gasoline (subject to certain specifications) which they sell at large distances from their own refineries. The companies which add manganese to gasoline have their refineries located in Southern California. These companies do not require the addition of manganese to exchanged gasoline which they sell in Northern California.[5,6]

A rough comparison of k_{veh} with ratios of manganese to lead in leaded gasoline will be made using XRF measurments on leaded gasoline sold by ARCO and Texaco in Southern California in May of 1986. The observed ratio of manganese to lead in leaded gasoline was 0.1 for Texaco and 0.12 for ARCO. Because together these two companies added only about three-eighths of the *statewide* total of lead in gasoline during this quarter,[10] it therefore seems likely that the average ratio of manganese to lead in gasoline over any large region of California should be considerably less than the value of 0.1 obtained for the average k_{veh} in southern California. It is not known whether this apparent difference has a real origin or whether it is an artifact of the apportionment model.

The values of the fitted intercept S (unnamed sources) are systematically negative. The average values of $S/\langle Mn\rangle$ are −0.07 for southern California sites, −0.06 for the Bay Area sites, and −0.04 for those in the San Joaquin-Sacramento Valley. These values are not large enough to affect the average apportionment significantly, even if they are subtracted entirely from one average source fraction. The negative values of S may occur because one or both of the source fractions of manganse increases faster than linearly with its tracer element. For example, if a linear regression is performed on a non-linear function of the type kx^n ($n > 1$), the fitted intercept will be negative and the fitted slope will exceed k. This may contribute to the high values of k_{veh} for southern California sites.

In summary, motor vehicles apparently make a significant contribution to levels of airborne manganese in southern California. Most of this is in fine particles, according to ratios of manganese levels to those of crustal tracers like silicon and titanium. The vehicular contribution may be significant at both source and receptor locations in Southern California. Elsewhere in California, the earth's crust makes the predominant contribution to airborne manganese, according to a statistical model of source apportionment.

Finally, it should be noted that according to Section 2254 of Title 13, Administrative Code of the State of California, it is legal to add manganese to leaded gasoline to be sold in California but illegal to do so to unleaded gasoline.

Acknowledgments

We are grateful for helpful assistance from Dr. Joseph DeVita regarding manganese content in gasoline, and to Michael Scheible for several helpful suggestions.

This report has been reviewed by the staff of the California Air Resources Board and approved for publication. Ap-

proval does not signify that the contents necessarily reflect the views and policies of the Air Resources Board, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

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PARTICULATE EMISSIONS

The amount of manganese which is exhausted from cars using 0.03125g Mn/gal as HiTEC®3000 is important when making estimates of airborne manganese concentrations. For this reason we measured airborne particulate on three models at 75,000 miles. We used the EPA particulate sampling techniques per CFR 86.110-82, 86.111-82, and 86.112-82. This tunnel technique is used primarily for diesel particulate studies. The tunnel and sampling system were cleaned and preconditioned using an unleaded fleet vehicle. Particulate emissions were measured for both the EEE and HiTEC®3000 cars in three model groups: Group E with the 1.9L engine, Group G with the 2.5L engine, and Group T with the 3.0L engine.

Particulate filters for each bag were analyzed for manganese content at Ethyl's Baton Rouge research center.

Particulate emissions for the nine EEE-fueled cars averaged 0.007g/mi and averaged 0.004g/mi for the nine HiTEC®3000 cars. Average manganese emission for the nine HiTEC® cars was less than 5.0 micrograms per mile, or about 0.40 percent of manganese input to the engine. Data for the 18 cars is shown in Table _____.

TABLE _____

PARTICULATE EMISSION
1975 FTP-CVS PROCEDURE

EEE Fuel		HiTEC® 3000 Fuel			
Car No.	g/mi. Total	Car No.	g/mi. Total	u g/mi. Mn	% Mn Exhausted
G-1	0.003	G3	0.004	4.1	0.38
G-2	0.008	G5	0.005	5.1	0.40
G-3	0.014	G6	0.004	4.4	0.34
E-2	0.005	E1	0.003	7.3	0.64
E-3	0.007	E5	0.002	3.1	0.28
E-4	0.007	E6	0.004	7.2	0.64
T2	0.010	T1	0.004	3.1	0.18
T3	0.006	T4	0.004	3.2	0.20
T6	0.004	T5	0.005	7.3	0.47

at 75,000 miles

Katy Mahaffy A-90-16 IV-H-1

AIR/WATER *pollution report* May 14, 1990

New Report Sparks Furor Over Alternative Fuels

Even though the House of Representatives is about to debate a bill to amend the Clean Air Act (see story, p. 147), the controversy over alternative fuels last week erupted anew. Sparking the furor was a new report showing that gasoline blended with ethanol would actually increase urban smog. But a trade group speaking for the grain-based additive immediately branded the finding as a "hoax" aimed at keeping the nation's air both dirty and hazardous in the name of profits.

The report, **Air Pollution Consequences of Using Ethanol-Gasoline Blends in Ozone Non-Attainment Areas**, was prepared for the American Petroleum Institute by the Sacramento, Calif.-based Sierra Research Group. Based on smog language passed by the Senate (A/WPR, April 2, 1990, p. 99), it found that "smog levels are increased by six percent when ethanol is added to gasoline," said Sierra senior partner Thomas Austin at a May 8 press conference.

The reason for this, said Austin, is that burning high-oxygen gasoline increases emissions of a primary ozone ingredient -- nitrogen oxide, or NO_x -- when engines are running. At the same time, elevating oxygen levels makes gasoline evaporate faster, and this makes fuel systems in idle automobiles release more hydrocarbons (HCs) -- the other ozone precursor besides ultraviolet light and hot weather.

Austin did not dispute that adding a high-oxygen additive to gasoline would help trim emissions of carbon monoxide (CO) "by slightly altering the air/fuel ratio of engines." He added that ethanol yields a "typical CO reduction of 25 percent." But "the effects of increased HC and NO_x emissions have been virtually ignored by the proponents of alcohol-gasoline blends," said Austin.

"Using the latest data developed by the Statewide Air Pollution Research Center of the University of California [Riverside]," Austin went on, "we calculated that HC emissions from evaporating fuel create 35 times more smog than CO emissions. Recent television ad campaigns [sponsored by grain giant Archer Daniels Midland] have claimed that carbon monoxide reductions can contribute to reduced smog levels. But our study showed that this is a seriously misleading claim."

When the Environmental Protection Agency first studied high-oxygen fuels in the 1970s, "the same problem with fuel evaporation was found," Austin said, adding, "The increased HC emissions were so large that EPA could not approve the use of "gasohol" under the Clean Air Act. But EPA management basically 'chickened out' rather than facing the political pressure [to approve] ethanol-gasoline blends." A "loophole" in the act that allowed the sale of gasohol, Austin stated.

While the Senate's air bill requires the sale of high-oxygen gasoline in regions with the highest CO levels, nowhere does it mandate ethanol. However, ethanol is alone among available additives that would meet the Senate's mandate for an oxygen level of 3.1 percent in a mix of 90 percent gasoline and 10 percent additive, Austin said. Adding another widely touted additive -- methanol -- would yield an oxygen level of 2.7 percent, he added.

But "in the hour and half we've had to study Austin's report, we determined that adding ethanol to gasoline would decrease ozone by 2 percent," said Eric Vaughn, president of the Renewable Fuels Association, at a press conference held immediately after API's. "Austin," said Vaughn, "is perpetuating a hoax. The Senate bill would allow up to seven types of fuel. It is absolutely inaccurate to say ethanol is the only fuel that would be allowed."

Nor does "API ever talk about all the 'bad actors' in gasoline that adding ethanol would take out," Vaughn went on. "Adding 10 percent ethanol would decrease emissions of xylene 8 percent, and xylene is the single worst 'actor' in gasoline. It forms ozone within hours," Vaughn argued. He added that many of the other ingredients in gasoline that ethanol would supplant -- like benzene and toluene -- are carcinogens. "So the only downside to ethanol," he stated, "are API's profits." And for API to "put out this report a week before the House debate," Vaughn added, "is shameful."

Chemical Company Asks EPA OK For Anti-Pollution Additive

The Richmond, Va.-based Ethyl Corp. last week said it has asked for Environmental Protection Agency approval of a fuel additive that will "reduce overall tailpipe emissions" while "raising octane" and trimming "the toxic aromatic" ingredients in gasoline, said Ray Wilkins Jr., president of the firm's Chemicals Group, at a press conference.

The additive, "HiTec 3000" has been used in Canada (as "MMT") for the last 10 years "with no harmful effects to the environment," Wilkins added. Ethyl requested EPA approval for "HiTec" in 1978 and 1981, but was turned down over worries it would damage vehicular pollution controls and increase emissions of hydrocarbons.

But "the news on catalytic convertors and other pollution controls is all good," said Gary Ter Haar, Ethyl's vice president of Health and Environment. However, with regard to hydrocarbon emissions, Wilkins admitted that "there is a seven percent increase" in emissions of the ozone precursor.

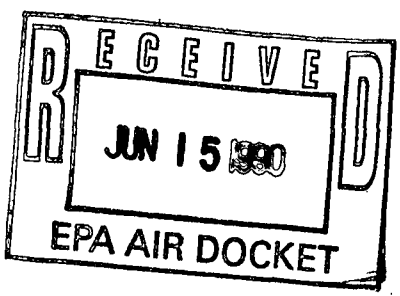
But "in the real world," there will be "no increase" at all, Wilkins added, explaining that "HiTec" would displace aromatic octane-enhancers that also are ozone precursors. At the same time, overall emissions of nitrogen oxide (NO_x), air toxics and carbon monoxide would be trimmed, and "we hope the big net benefit will win the day" when EPA reviews Ethyl's request for a fuel-additive waiver, Wilkins said.

Under 1977 amendments to the Clean Air Act, neither fuels or fuel additives may be marketed if they are "substantially different" from fuels already approved for motor vehicles unless EPA grants a waiver to Section 211 of the act.

"Our fleet-testing program demonstrates that use of 'HiTec 3000' in gasoline reduces automotive emissions of [ozone-precursor NO_x] by about 20 percent," he went on. "We estimate that if all automobiles use 'HiTec,' automotive NO_x will be reduced up to 644 million pounds annually by 1999." Vehicles are responsible for most NO_x emissions.

At the same time, "carbon monoxide is reduced by an average of 0.22 grams per mile." By reducing the aromatic content of gasoline "by 1.2 percent," moreover, gasoline refiners would have "the flexibility to [sustain] octane without raising vapor pressure."

EPA will have six months to repspond to Ethyl's request; if the agency does not respond in any way, the request will be approved by default.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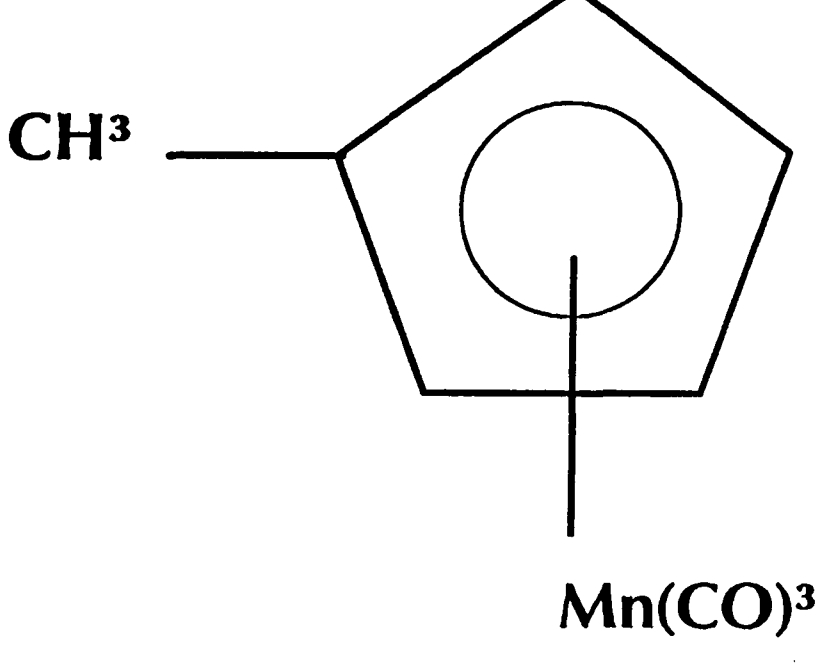
EPA AIR DOCKET

METHYLCYCLOPENTADIENYL MANGANESE TRICARBONYL

(MMT)



PROPERTIES OF ETHYL MMT

(METHYLCYCLOPENTADIENYL MANGANESE TRICARBONYL, COMMERCIAL)

Chemical Formula		$(CH_3C_5H_4)MN(CO)_3$
Molecular Weight		218.1
Manganese Content, min. % by wt.		24.4
Physical State		clear, straw colored liquid
Density, d_4^{20} g/ml		1.38
Viscosity at 20°C, cp		5.2
Refractive Index, N_D^{20}		1.588
	°C	°F
Freezing Point	-1	30
Boiling Point at 760 mm Hg	232	450
Flash Point (closed cup)	96	205
Solubility in:		
Water at 25°C		10 ± 2 ppm
Glycerine		± 5%
n-Hexane		miscible
n-Heptane		miscible
Isooctane		miscible
Toluene		miscible
Ethanol		miscible
Vapor Pressure at 70°F		0.05 mm Hg

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NeuroToxicology

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CONTENTS

NEUROTOXICOLOGY OF MANGANESE

Continued

ENHANCED AUTOXIDATION OF DOPAMINE AS A POSSIBLE BASIS OF MANGANESE NEUROTOXICITY*

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Autoxidation of dopamine as measured by its aminochrome formation at 480 nm was considerably potentiated by Mn^{++} in comparison to other biologically-important divalent cations such as Cu^{++}, Zn^{++}, Ni^{++},Ca^{++} and Mg^{++}. Effectiveness of autoxidation by metal ions tested was closely related to their redox potential. Manganese-enhanced autoxidation of dopamine was associated with increased generation of the free radicals O_2^-, H_2O_2, and $HO^{\cdot}$ as suggested by the inhibitory effects of superoxide dismutase, catalase and ethanol. Manganese, by enhancing the oxidation of dopamine, may augment considerably the production of neurotoxins emanating from this process and, under in vivo conditions, could be expected to contribute significantly to the neurodegenerative changes that accompany manganese dyskinesia in man.

INTRODUCTION

Since its first description by Couper in 1837, the phenomenon of manganese neurointoxication has intrigued researchers in a variety of disciplines. Probably a principal reason for such interest has been the remarkable similarity of manganism to the neuropathology, chemistry and clinical symptomatology of Parkinson's disease (Bernheimer et al., 1973; Mena et al., 1967). At autopsy, brains from manganese intoxicated patients, as well as from those with Parkinsonism, exhibit loss of melanin within the substantia nigra as a result of degeneration of this region and, to a lesser extent, the locus coeruleus in the rhinencephalon. Also, both types of disorder are typified by a dopamine deficiency in the caudate-putamen and of norepinephrine in the hypothalamus (Bernheimer et al., 1973). Clinically, manganism and Parkinsonism are associated with akinesia, rigidity and tremor, although dystonia is reputed to be a

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*Please send requests for reprints to Dr. Donaldson

*This manuscript is dedicated to the late and gifted pioneer of research in manganese neurotoxicity, Dr. G. Cotzias.

Neurotoxicology 2:53-64

more typical sign in manganism (Barbeau *et al.*, 1976). A considerable reduction in REM sleep characterizes both conditions which respond to treatment, at least initially, with L-dopa therapy.

Earlier attempts by one of us (Donaldson et *al.*, 1971; Donaldson et *al.*, 1974) to pinpoint the underlying neurochemical events in manganism led to the consideration that the striatal dopamine deficiency could be due to impaired transport of L-dopa across the neuronal membrane as a consequence of inhibition of the transport enzyme, Na^+ -K^+ -ATP'ase. However, Mn^{++} had no effect on the enzyme in microsomal preparations from rat-brain regions (Donaldson *et al.*, 1971). During the course of these experiments it was noted that intracerebroventricular (ICV) injection of manganese elicited rotational behavior in conscious, cannulated rats (Donaldson et *al.*, 1974), while bilateral intrastriatal injections of manganese produced a "freezing" or reserpine-like syndrome (Inoue *et al.*, 1975). Using similar routes of injection, the potent neurotoxin, 6-hydroxydopamine, (6-OHDA) elicits very similar behavior patterns in rats (Garattini and Samanin, 1977). Similarities in behavioral events evoked by such apparently diverse agents could be due to (a) the ability of Mn^{++} to promote the generation of 6-hydroxydopamine in nervous tissue or, (b) to another mechanism, common to both agents. Since 6-OHDA is widely believed to exert its cytotoxicity due to its ease of autoxidation as well as its capacity to liberate toxic oxidation byproducts (Sachs and Jonsson, 1975; Saner and Thoenen, 1971), it was considered that Mn^{++} may also exert its neurotoxicity by a similar or related mechanism. Graham (1978) and collaborators (Graham *et al.*, 1978) have recently demonstrated that cytotoxic products are indeed produced during autoxidation of dopamine and that the degree of autoxidation of a particular catecholamine was closely linked to its ability to inhibit cultured neuroblastoma cells. The present experiments were designed to explore the effects of manganese ions on dopamine autoxidation.

MATERIALS AND METHODS

The rate of autoxidation of dopamine was determined by measuring the increase in absorbancy of aminochrome formation in the presence and absence of Mn^{++} and other metal ions using a Spectronic 21 spectrophotometer at 480 nm. Experiments were performed using 5 ml of a 0.05 M Tris buffer at pH 7.8 with double-distilled deionised water. Dopamine HCl (Calbiochem) was employed at a concentration of 2.5×10^{-4}M unless otherwise indicated and the metal concentrations were 10 μM. In some experiments norepinephrine autoxidation was also determined using a similar procedure to that outlined. Incubation at 37°C was carried out in a shaking water bath. Concentrations of metals used in this investigation were designed to reflect the physiological condition as clearly as possible on the basis of previous studies on trace-metals in brain tissue (Donaldson *et al.*, 1973).

RESULTS

Effect of Various Metal Ions on the Autoxidation of Dopamine

As shown in Fig. 1, Mn^{++} was the most effective metal catalysing autoxidation of dopamine as measured by its aminochrome formation at 480 nm in Tris buffer, pH 7.8. In order of activity Mn > Cu > Ni > Zn > Mg > Ca. When the metals are ranked in order of their effectiveness in enhancing dopamine autoxidation, a striking parallel to their oxidation-reduction potentials is noted (Table 1). Thus, metals with high redox potential (Mn^{++}, Cu^{++}) exhibit greater efficacy in augmenting the autoxidation of the catecholamine than those displaying more electronegative potentials.

It was noted also (Table 2) that the pH of the reaction mixture influenced considerably the comparative efficacy of the metal ions Cu^{++} and Mn^{++} in augmenting dopamine autoxidation. At pH 7.4 for example Cu^{++} effects in stimulating the reaction were greater than those of Mn^{++}. However, this effect was directly related to the concentration of dopamine used (see later). As the dopamine concentration in the reaction mixture was raised it was found that Mn^{++} exerted greater stimulatory activity on the reaction. In all cases Mn^{++} activity was increased as the pH levels was raised above 7.4, possibly because of the formation of trivalent manganese in alkaline media. In physiological conditions manganese is thought to exist in this state (Venugopal and Luckey, 1978).

Effect of Increased Dopamine Concentration on the Catalytic Effectiveness of Cu^{++} and Mn^{++}

Fig. 2 shows the effect of increasing the substrate concentration on the Cu^{++} and Mn^{++} augmented autoxidation. Addition of Mn^{++} results in a corresponding increase in the absorbancy of the aminochrome formed in all concentrations of dopamine used. However, the Cu^{++}-stimulated autoxidation of the catecholamine becomes less effective as the concentration of dopamine is increased. Unlike Mn^{++}, Cu^{++} may form stable coordination complexes with dopamine, since this metal ion is chelated by the aromatic amino acid, 1-dopa (Corrodi et al., 1955). In other experiments (not shown) it was found that the oxidation-enhancing effects of Mn^{++} were directly related to concentration of the divalent cation. In alkaline solutions dopamine oxidation was found to be intrinsically sensitive to added Mn^{++} and the reaction is detectable with as little as 1 μM of Mn^{++}.

Metal Ion Stimulated Autoxidation of Norepinephrine

To determine if the stimulatory effects observed by Cu^{++} and Mn^{++} were restricted only to dopamine or if similar activity was exhibited with other catecholamines the autoxidation of norepinephrine (2.5×10^{-4}M in Tris buffer pH 8.6) in the presence of either Cu^{++} or Mn^{++} (10 μM) was determined. Manganese ions were found to stimulate the autoxidation process more effectively than Cu^{++} (Fig. 3).

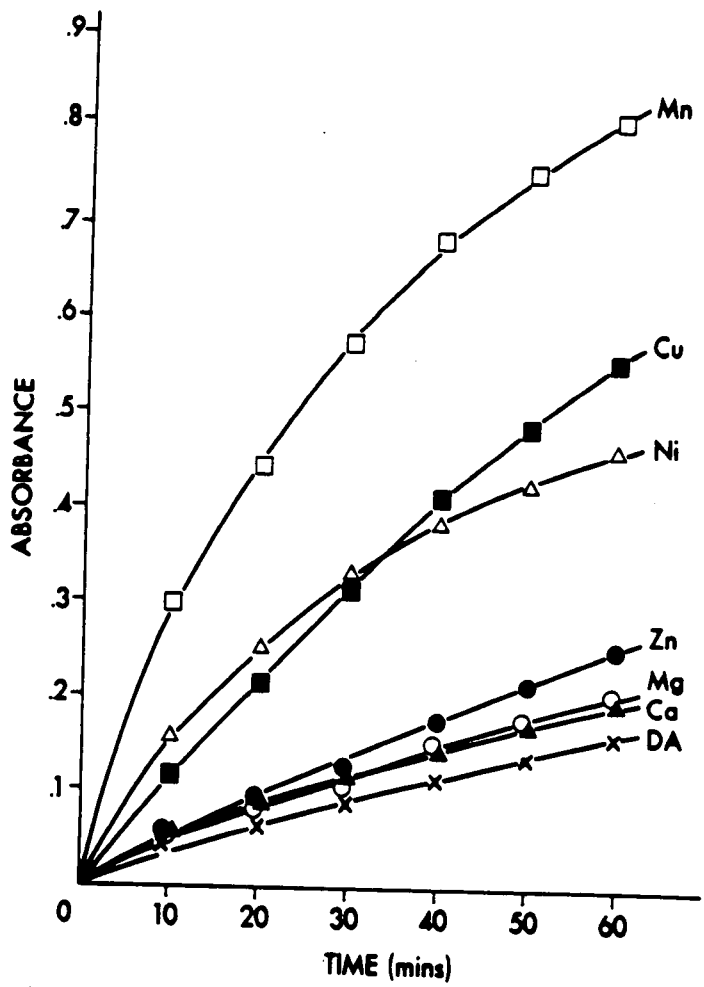

FIGURE 1. Metal ion catalysed autoxidation of dopamine. Dopamine (5×10^{-4}M) in Tris buffer, 0.05M, pH 7.8, containing metal ions (10 μM) was incubated in a shaking water bath at 37°C for various time periods. Absorbancy of aminochrome formed was recorded at 480 nm in a Spectronic 21 spectrophotometer. Control solutions containing Tris-dopamine only (DA) were treated similarly. Results shown are representative of 8 experiments.

Inhibition of Manganese-Enhanced Aminochrome Formation by Catalase

Estimation of H_2O_2 produced during the manganese-catalysed oxidation of dopamine was conducted by addition of catalase (Sigma, 14,000 U/mg protein) in varying amounts to incubation mixtures containing dopamine and Mn^{++} (50 μM). As shown in Fig. 4 enzyme addition resulted in a dose-dependent decrease in aminochrome formation. The amount of enzyme required for inhibition of the reaction at the elevated concentration of Mn^{++} employed in this experiment seems excessive, approximately 7,000 units

TABLE 1. Correlation of the Oxidation-Reduction Potential of Metals with Their Efficacy in Enhancing Dopamine Autoxidation

Metal	ΔAbsorbancy -60 min	Potential[1] E_0 (Volts)
$Mn^{+++} \rightleftharpoons Mn^{++}$	0.08	+ 1.51
$Cu^{++} \rightleftharpoons Cu^{+}$	0.55	+ 0.15
$Ni^{++} \rightleftharpoons Ni$	0.45	− 0.23
$Zn^{++} \rightleftharpoons Zn$	0.25	− 0.76
$Mg^{++} \rightleftharpoons Mg$	0.20	− 2.37
$Ca^{++} \rightleftharpoons Ca$	0.17	− 2.76

[1]Data obtained from Handbook of Chemistry and Physics, 59th ed., CRC Press, 1973.

being required for 50% inhibition. This could indicate the production of increased amounts of H_2O_2 were being generated during Mn^{++} oxidation since in other experiments using 10 μM of Mn^{++} about 50% inhibition was obtained with 2,000 units of the enzyme.

Inhibition of Manganese-Enhanced Aminochrome Formation by Superoxide Dismutase

Determination of superoxide anion, O_2^-, produced during the manganese-stimulated reaction with dopamine was determined by addition of superoxide dismutase (SOD) to reaction mixtures containing varying amounts of Mn^{++}. Mn^{++} addition led to increased dopamine oxidation, while O_2^-,

TABLE 2. Effect of Ph on Copper and Manganese-Stimulated Aminochrome Formation from Dopamine

pH	Increase in Absorbancy (% Control) Cu^{++}	Mn^{++}
7.2	200	120
7.4	220	150
7.8	240	380
8.0	280	450

Metal ions (10 μM) were added to beakers containing Tris 0.05M and dopamine 5×10^{-4}M at the various pH values indicated. Absorbancy at 480 nm was recorded following incubation at 37°C for 30 minutes. Control beakers contained similar amounts of Tris and dopamine only.

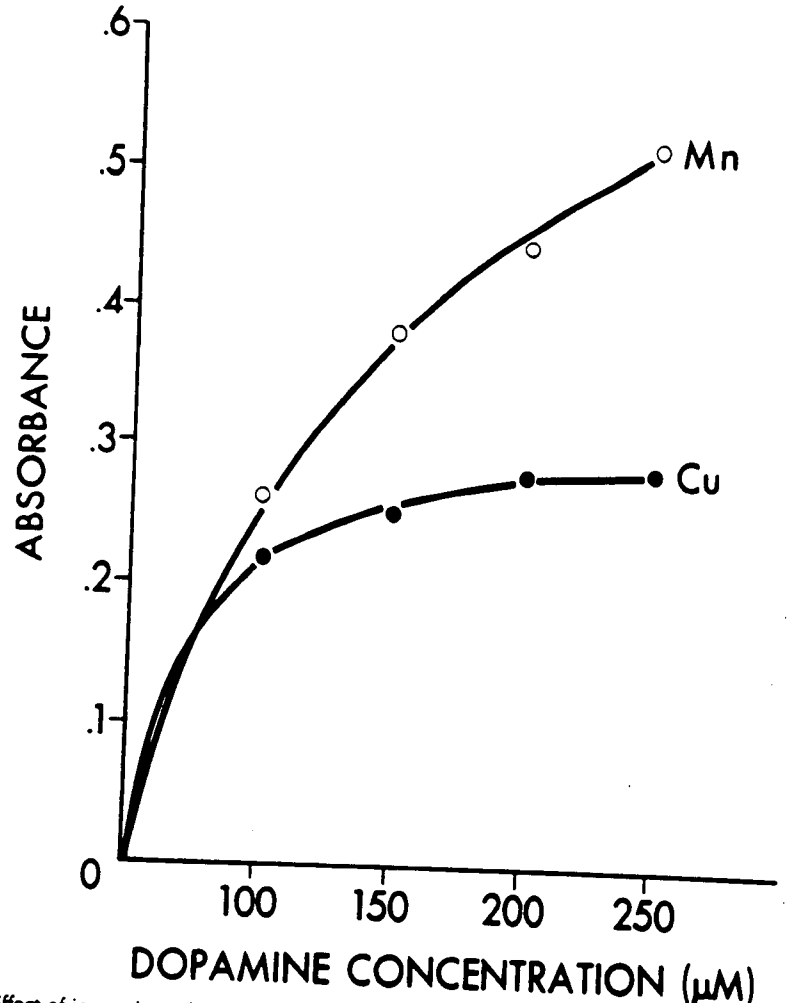

FIGURE 2. Effect of increasing substrate concentration on the Cu^{++} and Mn^{++}-catalysed autoxidation of dopamine. Cu^{++} or Mn^{++} (10 μM) was added to Tris buffer, 0.05M, pH 7.8, containing dopamine in amounts shown. Absorbancy of the aminochrome at 480 nm recorded following 30 min incubation in a shaking water bath at 37°C.

production, declined correspondingly. This effect of decreased enzyme inhibitory activity as Mn^{++} concentration was increased could be due to an inhibitory effect of the divalent cation itself on the enzyme protein. To rule out possibilities of cation antagonism between Mn^{++} and the Cu^{++}-Zn^{++} moieties of SOD, aliquots of the enzyme were incubated with Mn^{++} (100μM) in Tris buffer, 0.05M, pH 7.8 for 3 hr at 37°C. Samples of SOD incubated with buffer only were used as controls. Control and Mn^{++}-incubated SOD samples were dialyzed against triple-distilled water in the cold and tested for inhibition of dopamine autoxidation. As shown in Table 3, preincubation of the enzyme with Mn^{++} did not reduce its capacity to inhibit aminochrome formation from

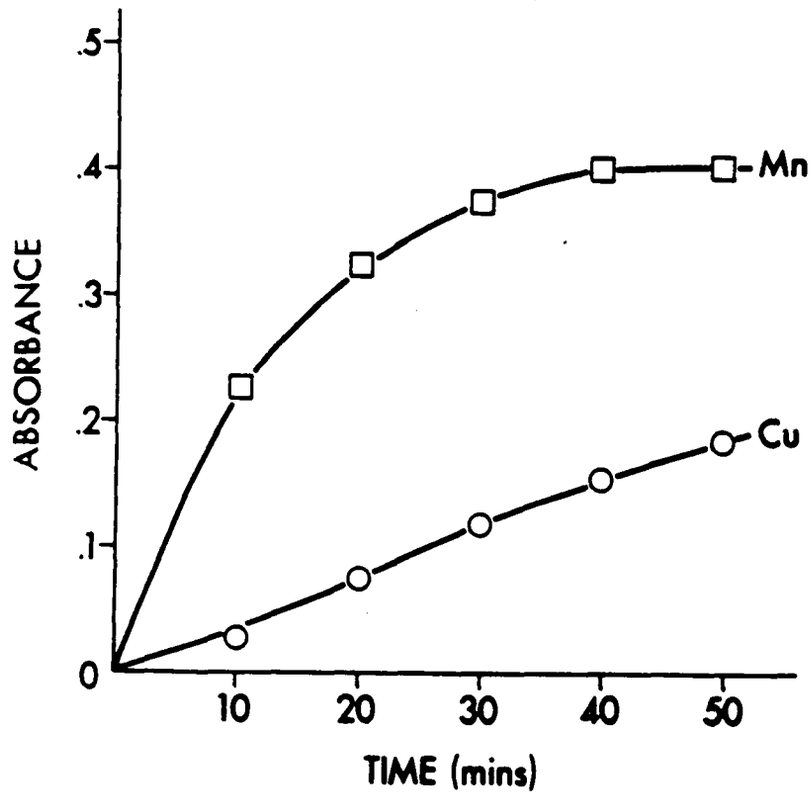

FIGURE 3. Cu^{++} and Mn^{++}-catalysed autoxidation of norepinephrine. Cu^{++} or Mn^{++} (10 μM) was added to Tris buffer, 0.05M, pH 7.8 containing l-norepinephrine (2.5×10^{-4}M). Absorbancy recorded at 480 nm following incubation at 37°C at time intervals shown.

dopamine, thus rendering unlikely any inhibitory interaction between the divalent cation and enzyme.

Inhibition of Metal-Catalysed Dopamine Autoxidation with Hydroxyl-Radical Trapping Agents

Detection of hydroxyl radicals produced during the metal-catalysed aminochrome formation from dopamine was determined using ethanol. The effectiveness of ethanol in suppressing the oxidation reaction (Table 4) is almost comparable to the order of the particular metal in promoting dopamine autoxidation, with Mn^{++} displaying greater susceptibility to inhibition by the hydroxyl radical scavenger. Thiourea was also an effective inhibitor of Mn^{++}catalysed oxidation but this may be due to the chelating properties of this agent rather than its ability to trap hydroxyl radicals. Sodium benzoate at elevated concentrations also inhibited the reaction.

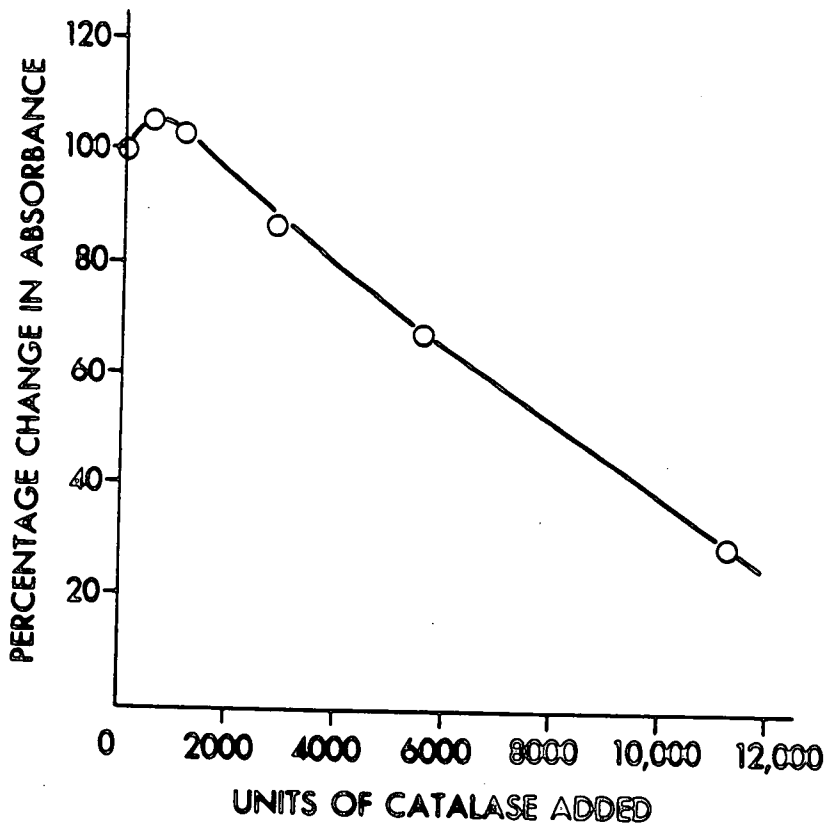

FIGURE 4. Catalase inhibition of Mn^{++}-catalysed autoxidation of dopamine. Catalase in amounts indicated was added to Tris (0.05M, pH 7.8) dopamine (5×10^{-4}M) solutions at start of reaction. Following incubation at 37°C 30 min absorbancy recorded at 480 nm. Tris-dopamine solutions incubated in absence of catalase served as controls. All solutions contained Mn^{++} (50 μM).

DISCUSSION

Potency of metal ions in promoting the autoxidation of dopamine to aminochrome was found to be closely related to the redox potential of the metals. Thus, Mn^{++} was the most effective ion in the catalysis of dopamine, and also is the most electropositive of the divalent ions studied. These results confirm and extend those of Gillette *et al.*, (1954, 1955) who earlier noted the remarkable effectiveness of Mn^{++} in stimulating catecholamine oxidation based on enhanced oxygen consumption in an alkaline reaction medium. Since aminochrome formation from dopamine was directly related to the Mn^{++} concentration used it is possible that at markedly-enhanced autoxidation rates there is a concomitant increase in free-radical production. The results of the addition of the scavenger for H_2O_2, catalase, would indicate that this is so since the amounts used for inhibition of the reaction appear excessive compared to those reported by other workers (Gillette *et al.*, 1954, 1955).

TABLE 3. Effect of Superoxide Dismutase on the Manganese-Enhanced Autoxidation of Dopamine

Manganese	Absorbance (480 nm) Percent/Control[1]
Control	100 (±4)
2.5	54 (±6)
5.0	69 (±8)
10.0	71 (±9)
15.0	78 (±6)
Dialysed SOD-Control	100 (±8)
Dialysed SOD-incubated with Mn^{++} (100 μM)	96 (±6)

[1]Data shown is the mean of 5-6 determinations (±SE) except for SOD dialysis (3 experiments).

Superoxide dismutase (Sigma, bovine blood, 3000 U/mg protein), 50 μg, was added at the start of the reaction to beakers containing Tris 0.05M, pH 7.8 and dopamine (2.5×10^{-4}M) with Mn^{++} amounts as indicated. Incubation at 37°C in a shaking water bath was conducted for 30 minutes when absorbancy (480 nm) was recorded. Each of the test samples containing Mn^{++} in the amounts listed were always incubated with a control sample containing similar amounts of Mn^{++} and other reactants but which lacked SOD. Thus, aminochrome formation in test beakers was related to its appropriately matched control. For comparative purposes the overall values were normalized to a common SE.

TABLE 4. Effect of Hydroxyl Radical Scavenging Agents on Metal-Catalysed Dopamine Autoxidation

Agent	Metal	Absorbance (480 nm) Percent/Control
Control	As per test sample	100 (±6)
Ethanol (0.25M)	Mn^{++}	73 (±8)
Ethanol "	Zn^{++}	82 (±7)
Ethanol "	Cu^{++}	88 (±6)
[illegible]	Ni^{++}	95 (±6)
Thiourea (10 mM)	Mn^{++}	72 (±6)
Sodium benzoate (0.5M)	Mn^{++}	80 (±5)

Agents used were added at the start of the reaction to beakers containing Tris 0.05M, pH 7.8 and dopamine (2.5×10^{-4}M) with metals (10 μM) listed. Incubation at 37°C in a shaking water bath was continued for 30 minutes and absorbancy at 480 nm recorded.

Values indicated are the mean of 3-4 experiments (±SE).

Each of the test samples containing metal ions as indicated were always incubated with a control sample containing similar amounts of metal ion and other reactants but lacking a scavenging agent. Aminochrome formation in test samples was thus related to its appropriately matched control. For comparative purposes [illegible] control values were normalised to a common SE.

Incremental Mn^{++} addition, while enhancing dopamine autoxidation, paradoxically led to an apparent decrease in generation of O_2. That Mn^{++} may react with the generated H_2O_2 itself to produce highly-reactive hydroxyl radicals cannot be discounted. This assumption is supported by the diminished effectiveness of the SOD in the presence of increasing Mn^{++} concentrations. The metal itself was found to have no effect on the enzyme activity per se. Kappus and Schenkman (1979) found that SOD was unable to inhibit Mn^{++}-stimulated epinephrine oxidation and suggested that Mn^{++} was chelated by the catecholamine. This explanation seems unlikely because Mn^{++} efficacy in catalysing dopamine autoxidation was enhanced by increasing dopamine content in the incubation medium, while Cu^{++}, which readily forms coordination complexes with catecholamines (Gillette *et al.*, 1954, 1955) exhibited diminished catalytic activity. The concentration of Mn^{++} found necessary by these workers for the non-enzymatic oxidation of epinephrine by Mn^{++} were about 200-fold those used in the present investigation for autoxidation of the structural analogue, norepinephrine. A plausible explanation for the ineffectiveness of SOD in inhibiting Mn^{++}-augmented dopamine autoxidation is that Mn^{++} is itself catalysing the dismutation of O_2^-. Such scavenging activity by the divalent ion would result in competition for the available O_2^- generated in the reaction, thus resulting in antagonism of the enzymatic dismutation by SOD. Such antagonism would be reflected in the apparent inability of the enzyme to effect disproportionation of the O_2^- produced in the presence of additive Mn^{++}. In this regard, there is evidence that, in the presence of sufficient water molecules, superoxide dismutase can bring about the oxidation of metal ions possibly as a result of initial proton-induced dismutation of O_2^- to products that effect the oxidation (Sawyer and Gibian, 1979). That Mn^{++} and SOD may compete for available O_2^- is supported by Kono *et al.*, (1976) who found that Mn^{++} was an effective inhibitor of O_2^--mediated reactions generated by either xanthine-oxidase or FMN light systems. They consider that such action by Mn^{++} was probably due to the oxidation of Mn^{++} to Mn^{+++} rather than to its effect on catalysing the disproportionation of O_2^-. The oxidation of Mn^{++} was accompanied by a doubling of H_2O_2 formation and a tripling of oxygen consumption.

In the present investigation the apparent ineffectiveness of SOD with increasing Mn^{++} concentration was not due to enzyme inactivation by the metal, since incubation with Mn^{++} (100 μM) failed to alter enzymatic activity. Accordingly, it can be anticipated on the basis of our results as well as those of Kono *et al.*, (1976) that oxidation of devalent manganese to the trivalent form is due to the affinity of Mn^{++} for the superoxide anion generated during autoxidation. Trivalent manganese is the biologically-active form in mammals and in this form is more avid than Mn^{++} towards chelation in biological systems owing to the smaller Mn^{+++} ionic radius (Venugopal and Luckey, 1978). Alkalinity can influence the valency state of metals and Mn^{+++} formation is favored in alkaline medium. A pH of 7.8 as used in our experiments was found to strongly increase the catalytic activity of manganese in stimulating dopamine oxidation compared to pH levels of 7.4 and lower. Alteration of the pH did not appreciably alter the oxidation efficacy of Cu^{++}, although the latter ion's catalytic activity was suppressed by increasing dopamine concentration.

As noted earlier in the Introduction, both Mn^{++}, as well as tht potent neurotoxin, 6-hydroxydopamine, display similar behavioral (Inoue *et al.*, 1975; Garattini and Samanin, 1977) and neurochemical (Donaldson, In preparation) properties when injected into rat brain. Present results would indicate that these effects may be explicable on the basis of both agents possessing a [illegible] mechanism of action. For example, the ability of 6-OHDA and [illegible] to undergo autoxidation with concomitant production of by-products which are neutoxic has been reported (Graham, 1978; Graham *et al.*, 1978). Manganese, by enhancing the oxidation of dopamine, could thus be expected to augment considerably the cytotoxins emanating from this process, and, under *in vivo* conditions, could be expected to contribute significantly to the neurodegenerative process. Since neuromelanin formation can occur non-enzymatically (Rodgers and Curzon, 1975), by formation principally from dopamine (Das *et al.*,1978), and in view of the potentiation of dopamine oxi[illegible] by Mn^{++} in the physiological levels used in the present investigation, it [illegible]ivable that Mn^{++} neurotoxicity arises predominantly through its enhancement of catecholamine autoxidation in regions richly innervated with catecholaminergic neurons. This would likely occur in discrete brain regions, like the substantia nigra where melanin granules predominate and dopamine cell-bodies are located.

Depigmentation due to loss of melanin, as well as neuronal degeneration of this region, is a feature of both manganism and the associated desorder, Parkinsonism (Bernheimer *et al.*, 1973). In manganese neurointoxication such degeneration could be induced possibly as a result of the free radicals pro[illegible]ing potentiated dopamine autoxidation by this divalent ion. Alternati[illegible]se effects could arise by lipid peroxidation due to initiation of this process by increased amounts of the free radicals generated. Since the basal ganglia is a particularly vulnerable region in manganese intoxication, the ability of Mn^{++} to undergo oxidation to Mn^{+++} in this brain region of intense oxidative activity could be anticipated to further facilitate neuronal degeneration since oxidation of Mn^{++} is accompanied by a tripling in oxygen consumption and in a doubling of H_2O_2 formation (Kono *et al.*, 1976).

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Epidemiological Survey Among Workers Exposed to Manganese: Effects on Lung, Central Nervous System, and Some Biological Indices

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A cross-sectional epidemiological study was carried out among 141 male subjects exposed to inorganic manganese (Mn) in a Mn oxide and salt producing plant (mean age 34.3 years; duration of exposure, mean 7.1 years, range 1–19 years). The results were compared with those of a matched control group of 104 subjects. The intensity of Mn exposure was moderate as reflected by the airborne Mn levels and the concentrations of Mn in blood (Mn-B) and in urine (Mn-U). A significantly higher prevalence of cough in cold season, dyspnea during exercise, and recent episodes of acute bronchitis was found in the Mn group. Lung ventilatory parameters (forced vital capacity, FVC; forced expiratory volume in one second, FEV_1; peak expiratory flow rate, PEFR) were only mildly altered in the Mn group (smokers) and the intensity and the prevalence of these changes were not related to Mn-B, Mn-U, or duration of exposure. There was no synergistic effect between Mn exposure and smoking on the spirometric parameters. Except for a few nonspecific symptoms (fatigue, tinnitus, trembling of fingers, increased irritability), the prevalence of the other subjective complaints did not differ significantly between the control and Mn groups. Psychomotor tests were more sensitive than the standardized neurological examination for the early detection of adverse effects of Mn on the central nervous system (CNS). Significant alterations were found in simple reaction time (visual), audioverbal short-term memory capacity, and hand tremor (eye-hand coordination, hand steadiness). A slight increase in the number of circulating neutrophils and in the values of several serum parameters (ie, calcium, ceruloplasmin, copper, and ferritin) was also found in the Mn group. There were no clear-cut dose-response relationships between Mn-U or duration of Mn exposure and the prevalence of abnormal CNS or biological findings. The prevalences of disturbances in hand tremor and that of increased levels of serum calcium were related to Mn-B. The response to the eye-hand coordination test suggests the existence of a Mn-B threshold at about 1 μg Mn/100 ml of whole blood. This study demonstrates that a time-weighted average exposure to airborne Mn dust (total dust) of about 1 mg/m^3 for less than 20 years may present preclinical signs of intoxication.

Key words: manganese, human lung effects, CNS, biological indices

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INTRODUCTION

The two main target organs following long-term exposure to Mn dust are the central nervous system (CNS) and the lungs. The clinical manifestations have been well characterized by epidemiological studies among workers chronically exposed to very high levels of airborne Mn (for reviews: WHO, 1981; US-EPA, 1984). Severe chronic Mn intoxication is associated with neurobehavioral symptoms and neurological signs characteristic of the extrapyramidal syndrome [Rodier, 1955; Schuler et al, 1957; Chandra et al, 1974] most likely due to functional or degenerative changes in the central dopaminergic system [Cotzias et al, 1971, 1976; Bernheimer et al, 1973]. In the early stage, the tremor associated with chronic Mn intoxication is frequently an intentional tremor and not a resting tremor characteristic of Parkinson's disease [Klawans et al, 1970]. At the advanced stage of manganism, autonomic disturbance may also occur (eg, increased salivation and sweating). An increased incidence of pneumonia and/or bronchitis has also been reported in workers with occupational Mn exposure [Lloyd-Davies, 1946; Wasserman and Mihail,1961; Saric and Lucic-Palaic, 1977] and in people living around factories producing Mn alloys [Suzuki, 1970; Nogawa et al, 1973].

Manganese toxicity exhibits great interindividual variability and the lowest exposure level at which early effects on the CNS and the lungs may occur is still unknown. Clinical signs of chronic Mn intoxication have seldom been reported at exposure levels below 5 mg/m^3 [Saric et al, 1977; Chandra et al, 1981; Tanaka and Lieben, 1969; Sabnis et al, 1966]. An international group of experts [WHO, 1981] concluded that "signs of adverse effects on the CNS may occur at manganese concentrations in air ranging from 2 to 5 mg/m^3." US-EPA [1984] stated that epidemiological studies in humans indicate effects on the respiratory system at levels below 1 mg/m^3, whereas studies of effects on the CNS below this level are equivocal or negative.

The evaluation of the dose(exposure)-response relationship is complicated by the fact that good biological indicators of Mn exposure are not presently available. There is some indication that on a group basis Mn in urine reflects mainly current exposure [Tanaka and Lieben, 1969; Smyth et al, 1973; Roels et al, 1987], whereas Mn in blood seems more influenced by the body burden of Mn [Keen et al, 1983; Roels et al, 1987].

The present article describes the results of a cross-sectional epidemiological study among workers exposed to Mn in a plant producing Mn oxides and salts from Mn ore. Up to now, most of the epidemiological studies were designed to detect clinical manifestations of Mn intoxication. However, once neurological signs and symptoms are present, they tend to persist and may even worsen after cessation of exposure [Barbeau et al, 1976]. Therefore, it is very important to detect adverse Mn effects when the disease is still in a preclinical and possibly reversible stage of development. In the present study, in addition to a standardized neurological examination, several psychomotor tests (hand tremor, short-term memory, and simple reaction time), designed to detect any early effects of Mn on CNS were given. We also measured the peripheral serotonin content and platelet monoamine oxidase (MAO) activity. Since a few human studies and several experimental investigations

[Chandra et al, 1973, 1974; Jonderko et al, 1971; Banta and Markesbery, 1977; Mena et al, 1969, 1974; Thomson et al, 1971; Panic, 1967; Gubler et al, 1954] suggest the existence of possible interactions between Mn and the metabolism of other metals (eg, calcium, copper, iron), we determined the serum level of calcium, ceruloplasmin, and ferritin. The ventilatory status of the workers was also assessed.

SUBJECTS AND METHODS

Study Population and Study Design

The Mn factory is one of the world's major producers of manganese oxides (Mn_3O_4, MnO_2) and salts (sulfate, carbonate, nitrate) using concentrated ores. Its total workforce consisted of about 150 male employees, of whom 141 Mn-exposed subjects were examined [Roels et al, 1987]. A group of 104 male workers from a nearby chemical plant was recruited as the control group. Control and Mn-exposed workers were matched for socioeconomic status and background environmental factors. Both groups had comparable workload and workshift characteristics. At the time of the survey, all the workers were clinically in good health.

Biological analyses at the time of the survey and information gathered by a questionnaire ascertained that 1) the control and Mn-exposed workers were neither currently nor previously excessively exposed to lead, cadmium, mercury, or solvents. At the time of the survey, the individual values of lead (PbB) and zinc-protoporphyrin (ZPP) in blood and of cadmium (CdU) and mercury (HgU) in urine were normal, ie PbB < 35 μg/dl, ZPP < 2.5 μg/g Hgb, CdU < 2 μg/g creatinine, and HgU < 5 μg/g creatinine; and 2) the exposed workers had been exposed to manganese dust for at least 1 year prior to the study, whereas the controls had never been occupationally exposed to manganese.

To standardize as much as possible the performance of all the tests with respect to the workshift schedules in the two plants, we carried out the clinical examination once a week (on Tuesday), while the workers were on day shift. Five control and seven Mn-exposed workers were examined each week, two controls and four exposed in the morning and three subjects of each group in the afternoon. The survey was conducted by three well-trained investigators who were responsible for the same part of the examinations throughout the whole study period. The examinations included a questionnaire, a standardized neurological examination, lung function tests (ventilatory parameters), psychomotor tests, and sampling of blood and urine for biological analyses. These were carried out in separate rooms of the medical department of the control plant. The workers removed their work clothing and showered before the clinical examinations. The overall examination period took approximately 90 min per subject, including the time needed to learn how to perform the tests.

Mn Exposure

No monitoring data were available to characterize the past pollution of the work environment by manganese. It should however be pointed out that the Mn plant started production in 1964 and that the production processes and the different halls have not subsequently undergone environmentally significant changes. Since 1965, the production of MnO_2 increased systematically: 1965, 440 metric tons (mt); 1970, 2,300 mt; 1975, 8,900 mt; 1980, 18,600 mt, and 1981, 22,000 mt.

The time-weighted average concentration of total airborne manganese dust during the survey ranged from 0.07 to 8.61 mg/m^3, with the overall mean and median of 1.33 and 0.97 mg/m^3, respectively. The geometric mean and the 95th percentile value amounted to 0.94 and 3.30 mg/m^3, respectively [Roels et al, 1987].

Questionnaire

The day before the examination, each worker answered a self-administered questionnaire which was checked and completed the next day during the interview with the examiner, who also consulted the worker's medical file.

In the first part, information was gathered on age, weight, height, educational level, occupational and health history, actual and previous smoking habits, and coffee and alcohol (mainly beer) consumption. The examiner put special emphasis on details regarding estimated current and/or past exposure to mercury, lead, cadmium, and solvents.

In the second part, twenty-five questions (yes or no answers) were asked in order to detect symptoms mainly related to the nervous system, such as decreasing memory, fatigue, headache, depressive feelings, sleep disturbances, cramp in muscles, paresthesia, tinnitus, dizziness, perspiration, salivation, tremor, irritability, anxiety, changes in sexual desire, and impotence.

In the third part, twenty questions (adapted from the British Medical Research Council (MRC) questionnaire on chronic bronchitis [1965] used in the European Community) were asked to detect complaints related to the respiratory system.

Lung Function Test

Maximal expiratory flow-volume curves were obtained by displaying on a storage oscilloscope the flow rate, measured with a Fleisch 4 pneumotachograph, versus expired volume obtained by integration of flow rate. Each worker performed at least four forced vital capacity (FVC) maneuvers (in standing position). An electronic apparatus [Fesler et al, 1975] enabled the digital readout of the following indices: FVC, forced expiratory volume in one second (FEV_1), and peak expiratory flow rate (PEFR). The maximal expiratory flow rate at 50% and at 75% of FVC ($\dot{V}\,max_{50}$ and $\dot{V}\,max_{75}$) were measured from polaroid photographs of the flow-volume curves. No rejection criteria were defined but for each subject only the highest values were taken into account for data analysis. For each subject, the observed values of the ventilatory parameters were expressed as a % of the predicted values which were calculated on the basis of his age, weight, and height. The prediction equations for an adult male were obtained from the following sources: FVC and FEV_1 from ECCS [1983] and PEFR, $\dot{V}max_{50}$ and $\dot{V}max_{75}$ from Bass [1973].

Standardized Neurological Examination

A single, blind neurological examination was performed on each subject in order to detect early signs of Parkinsonism. In this examination, particular attention was paid to pronation-supination of wrist, hand oscillation, thumb-index position, cog-wheel phenomenon, rigidity of neck and trunk, balancing of arms and tremor of fingers during walking, drawing, and handwriting, and masklike facies.

Psychomotor tests

Simple reaction time. Simple reaction time (visual) was measured electronically with a chronoscope (EAP, Issy-les-Moulineaux, France) as described previously

[Roels et al, 1985]. The task lasted 8 min in order to assess the eventual effect of fatigue on the subject's vigilance. The reaction time is expressed in 10^{-2} sec. Sporadic false reactions (longer than 0.8 sec) were not recorded. The mean reaction time and standard deviation were calculated for each subject after 2, 4, 6, and 8 min by averaging the results of 30 measurements made during each period of 2 min.

Short-term memory. Each subject performed an audioverbal short-term memory test, known as the 15-word test of Rey. This test comprises a recall task (five trials) and a recognition task (presentation of a 1-min story). The execution and the calculation of the memory scores are described in detail elsewhere [Roels et al, 1985].

Hand tremor. The apparatus of Bize (EAP, Issy-les-Moulineaux, France) offers two tests particularly designed to evaluate hand tremor, namely the orthokinesimeter test with the hand in a dynamic condition for testing eye–hand coordination and the hole tremormeter test in which the hand remains in a stationary position for testing hand steadiness. Intelligence and combination activities are minimally involved in both tests, so that performances are not likely to be influenced by the educational level of the subject. Details about the apparatus, the execution of those two tests, and the calculation of the tremor scores are explained elsewhere [Roels et al, 1982].

Biological Analyses

Blood and urine sampling. A sample of venous blood (25 ml) and a spot-urine sample (50 ml) were collected on the day of the clinical examination. Standardized syringes, tubes, and urine containers were used and they were previously checked for lack of heavy metal contamination. Blood samples were divided as follows: 2 ml in a tube (Sarstedt Monovette, EDTA-K) for routine hematological analyses, 8 ml in a polypropylene tube (containing 0.1 ml EDTA-Na_2 10%, w/v) for the assay of platelet monoamine oxidase (P-MAO) acitivity, 10 ml in a polystyrene tube (containing 0.1 ml EDTA-Na_2 10%, w/v) for the determination of blood serotonin and blood manganese, with the remainder used for serum collection. All tubes were kept overnight at 4°C and then analyzed. Immediately after the urine samples were obtained, an aliquot (4 ml) was poured into a tube and stored at −20°C until analysis of retinol-binding protein (RBP-U) and albumin. The remainder of the urine sample was kept at 4°C for further analysis.

Platelet monoamine oxidase activity (monoamine: oxygen oxidoreductase (deaminating) EC 1.4.3.4). MAO in human platelets (P-MAO) exhibits characteristics of the type B form with substrate specificity for β-phenyl-ethylamine and benzylamine [Tipton and Della Corte, 1979]. MAO activity was assayed in triplicate on platelet-rich plasma [PRP] utilizing kynuramine (Kynuramine.diHBr purchased from Sigma Chemical Co.) as the substrate [McEntire et al, 1979]. The assay is based on the appearance of 4-hydroxyquinoline (4-HOQ), a fluorescent product of the oxidative deamination of kynuramine. The fluorescent intensity was measured in 1-cm quartz curvettes using a spectrofluorimeter Aminco SPF-500 (American Instrument Company, Silver Spring, MD) at the following settings: excitation 315 nm, emission 380 nm, band pass 5 nm. P-MAO activity was calculated from a standard curve of 4-HOQ (4-hydroxyquinoline.$3H_2O$, 98%, Aldrich-Europe, Beerse, Belgium) and expressed in nmol 4-HOQ produced per h by 10^8 platelets. The number of platelets in PRP preparation was determined with a Hemalog 8 (Technicon) and showed that more than 85% of the platelets were recovered from whole blood.

Serotonin in blood. In blood, 95% of serotonin is found in the platelets. Whole blood serotonin was determined fluorimetrically (in triplicate) according to the three-step procedure of Yuwiler et al [1970] for the extraction of serotonin. The final mixture was measured in 1-cm quartz curvettes using a spectrofluorimeter Aminco SPF-500 at the following settings: excitation at 295 nm, emission at 540 nm, band pass 10 nm. The concentration was calculated from a standard curve of serotonin (serotonin creatinine sulfate monohydrate, Aldrich-Europe, Beerse, Belgium) and expressed in three ways, ie, in ng/ml whole blood,in ng/10^8 platelets, and in ng/μl of platelets.

Manganese in blood and in urine. The concentration of manganese in blood (Mn-B) and in urine (Mn-U) was determined in duplicate by flameless atomic absorption spectrometry after manganese has been chelated with cupferron and extracted with MIBK [Buchet et al, 1976]. A Perkin-Elmer Model 420 equipped with a deuterium background corrector and a HGA-76B atomizer unit were used. The method is directly applicable to urine but the determination of blood manganese required a preliminary wet digestion of 2 ml whole blood. The analyses were performed with the use of internal standards.

Serum analyses. Calcium in serum was analyzed by flame atomic absorption spectrometry using a Varian Techtron, Model 1100: 0.1 ml serum was diluted with 9.7 ml demineralized water and then supplemented with 0.2 ml of a 5% La^{3+} solution [Perkin-Elmer Corporation,1971]. Copper in serum was measured by flameless atomic absorption spectrometry using a Perkin-Elmer 305 and a HGA-74 atomizer unit: an aliquot of 0.1 ml serum was acidified with 0.9 ml HNO_3 0.05 N and another 0.1 ml of serum was supplemented with 0.5 ml HNO_3 0.05 N and 0.4 ml of a standard solution (in HNO_3 0.05 N) containing 0.2mg Cu/liter [Perkin-Elmer Corporation, 1971]. Ceruloplasmin, ferritin, and β_2-microglobulin in serum were determined by a nonisotopic immunoassay based on latex particle agglutination [Bernard and Lauwerys, 1983].

Urine analyses. The renal function was evaluated by measuring α-N-aminoaciduria using picrylsulfonic acid as reagent [Bernard et al, 1976], and specific proteins like albumin and retinol-binding protein in urine using the above mentioned latex particle agglutination technique. Creatinine in urine was determined according to Jaffe's picrate method [Henry, 1965].

Statistical Analysis

For comparison of mean values between control and Mn-exposed groups, the two-tailed Student t test was used. Differences in cumulative frequency distributions between control and Mn-exposed groups were ascertained by a $2 \times n$ χ^2 test (n = number of classes of at least 5). The prevalences of symptoms (lung, CNS) or of "abnormal" values of spirometric, CNS, or biological parameters were compared between the groups using the Yates corrected 2×2 χ^2 test. For the study of the dose-response relationships, the total study population was subdivided into four subgroups and differences in the prevalences of "abnormal" results were ascertained by a 2×4 χ^2 test. Unless otherwise stated, for each parameter, the results lower than the 5th or higher than the 95th percentile value in the control group were considered "abnormal." Analyses of variance were performed in order to ascertain possible effects of age, duration of exposure, smoking habits, or Mn exposure on certain variables. Pearson's correlation coefficients (simple and partial) were calcu-

transformation of non-Caussian distributions). For all tests, $P \leqslant 0.05$ was considered as the level of statistical significance.

RESULTS

Characteristics of Control and Mn-Exposed Groups

Table I shows some characteristics of the control and Mn-exposed groups. The controls were on the average 4 years older and 3 cm taller than the Mn-exposed workers. The duration of exposure to Mn ranged from 1 to 19 years with a mean of 7.1 years. Coffee and beer consumption was comparable between the control and Mn-exposed groups and there was no statistically significant difference (2×2 χ^2 test) between the number of workers in both groups consuming these beverages. The smoking habits reported in the control and Mn-exposed groups are summarized in Table II. The proportion of nonsmokers was rather low, ie, 28% and 20% in control and Mn-exposed groups, respectively. The number of current smokers and ex-smokers combined did not differ significantly (2×2 χ^2 test) between the control and Mn group. When considering the current and ex-smokers separately, slightly different smoking characteristics were found between control and Mn workers. The integrated cigarette smoking index (number of cigarettes per day × years of smoking) for the current smokers averaged 380 cig per day × yr in the controls versus 269 cig per day × yr in the Mn workers, whereas in the ex-smokers it was 410 versus 479 cig per day × yr, respectively. For the current and ex-smokers combined, the integrated cigarette smoking index averaged somewhat higher in the controls (390 cig per day × yr) than in the Mn-exposed workers (300 cig per day × yr). The number of subjects inhaling smoke was not significantly different (2×2 χ^2 test) between control (89%) and Mn workers (85%). With respect to educational level, the number of subjects with elementary education was 26 (25%) and 37 (26%) in the control and Mn-exposed group, respectively. The remaining subjects had at least junior high school education.

The internal exposure to manganese was estimated by the determination of manganese in blood and in urine. The results are presented in the previous paper

TABLE I. Characteristics of the Study Population

	Control (n = 104)				Mn-exposed (n = 141)			
	Mean	SD	Median	Range	Mean	SD	Median	Range
Age (years)	38.4[c]	11.3	36	19–58	34.3	9.6	33	19–59
Weight (kg)	78.7	9.6	78	54–101	76.2	12.9	75	46–130
Height (cm)	174.3[c]	6.8	174	160–197	171.3	6.4	171	155–187
Years of Mn exposure	—	—	—	—	7.1	5.5	6	1–19
Coffee[a]								
Cups/day	6.5	4.2	5	1–20	6.4	4.8	5	1–30
Years	20.5	11.7	20	3–47	17.9	10.6	16	1–50
Beer[b]								
Glasses/day	2.5	2.0	2	1–10	3.1	2.3	3	1–12
Years	18.1[c]	10.1	15	4–40	15.0	8.5	15	2–35

[a] Number of subjects drinking coffee: control, n = 96; Mn-exposed, n = 135.
[b] Number of subjects drinking beer: control, n = 74; Mn-exposed, n = 111.
[c] Values significantly higher in control group ($P < 0.05$).

group than in the control group, with significant differences for the 5th recall trial (11% with abnormally low score) and for the sum of the five trials after 30 s recall time (14% with abnormally low score).

Hand tremor. The overall evaluation of the orthokinesimeter parameters (mean values and cumulative frequency distributions) indicated that the eye-hand coordination in the Mn group was significantly less precise and more uncertain than that in the control group. The cumulative frequency distribution of four of the seven parameters measured, namely percentage precision, percentage imprecision, uncertainty, and inexactitude are presented in Figure 2. In the Mn group, the prevalences of abnormal score values for the eye-hand coordination parameters ranged from 12 to 14% and differed significantly (χ^2 test, $p < 0.05$) from those found in the control group (4–5%).

The average score values of the hole tremormeter test were invariably higher in the Mn group than in the control group, suggesting a decreased hand steadiness in the Mn group. This is illustrated in Figure 3 by the cumulative frequency distributions of the scores recorded for the holes Nos. 4, 5, and 6 which are the most

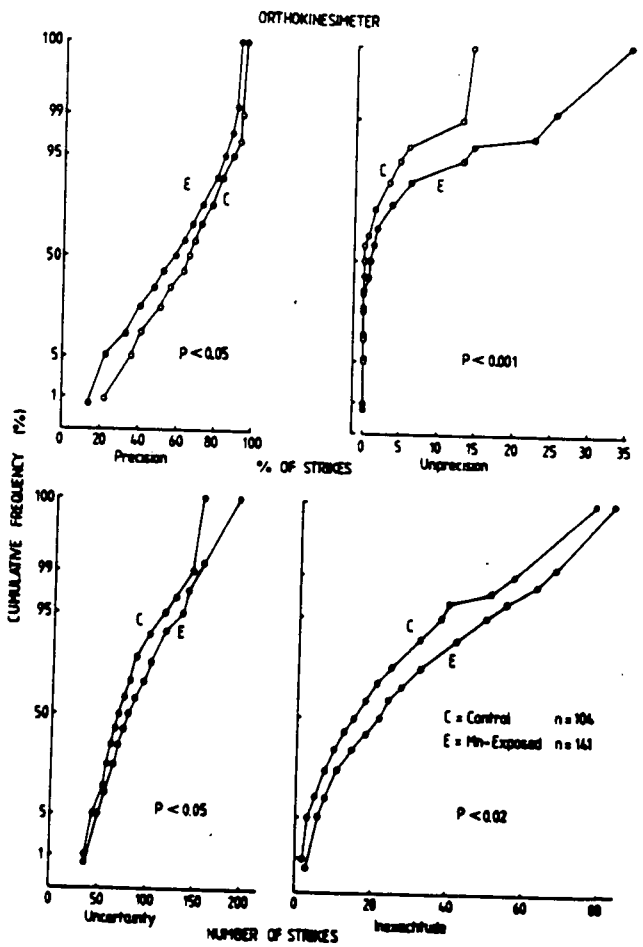

Fig. 2. Cumulative frequency distributions of orthokinesimeter parameters (eye-hand coordination) in control and Mn-exposed workers.

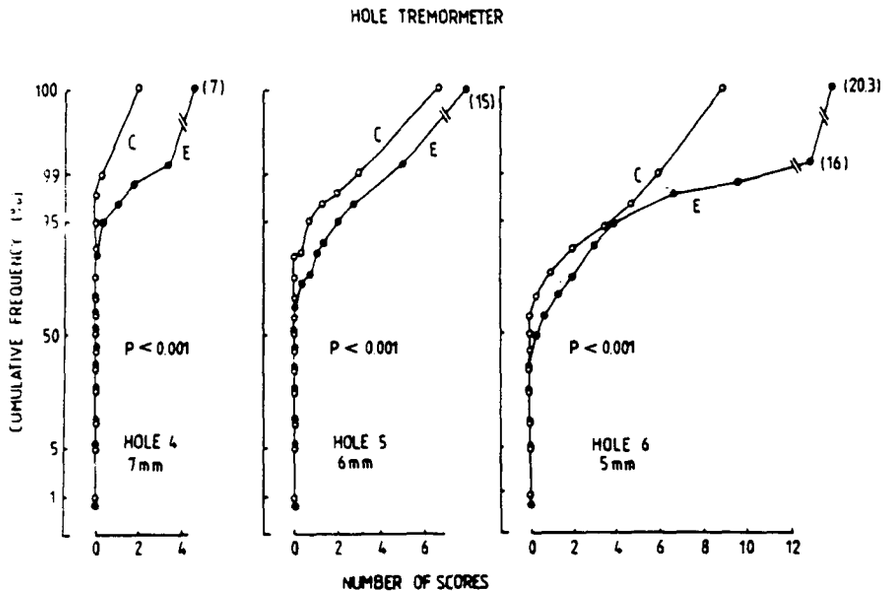

Fig. 3. Cumulative frequency distributions of hole tremormeter parameters (hand steadiness) in control and Mn-exposed workers.

discriminating ones. The differences between control and Mn-exposed workers became less pronounced for the holes Nos. 7, 8, 9, and 10, while the cumulative frequency distribution of the sum of the scores for the holes Nos. 3 to 10 was only marginally shifted to higher values in the Mn group. For hole No. 3 (8 mm), all the control workers had a zero score, whereas in the Mn group 7 workers could not perform faultlessly. The prevalence of abnormally elevated scores in the hand steadiness test was significantly higher (χ^2 test, $p < 0.01$) in the Mn group than in the control group for the holes Nos. 4 (12 vs 2%) and 5 (19 vs 5%).

Biological Parameters

The mean values of the hematological results were normal and did not differ between the control and Mn-exposed workers, except for the white blood cell (WBC) counts which were significantly higher (t test, $p < 0.001$) in the Mn group. This increase in WBC counts is entirely attributable to an increased number of neutrophils. The prevalence of results above the 95th percentile value of the control group amounted in the Mn group to 19% for WBCs and to 21% for neutrophils. Since there is evidence that smoking may increase the number of circulating WBCs [Baulande and Gérodias, 1982], we have looked at the effect of smoking and Mn exposure separately. The total study population was divided into the same four subgroups as for the statistical analysis of the spirometric parameters. A two-way analysis of variance revealed significant but independent effects of both smoking and Mn exposure on the number of circulating WBCs (smoking: F^1_{241} ratio = 12.81, $p < 0.001$; Mn exposure: F^1_{241} ratio = 11.4, $p < 0.001$) and neutrophils (smoking: F^1_{241} ratio = 10.63, $p < 0.01$; Mn exposure: F^1_{241} ratio = 13.5, $p < 0.001$). WBC and neutrophil counts averaged about 10% higher in the control smokers when compared to the control nonsmokers, while in the Mn-exposed smokers they were respectively 17 and

occurrence of acute and/or chronic bronchitis could not explain the higher WBC and neutrophil counts in the Mn group. The number of WBCs or neutrophils in the control as well as in the Mn group were not significantly different between subjects with or without signs of bronchitis. Furthermore, in the subjects without signs of bronchitis (84 in the control and 84 in the Mn group), the WBC and neutrophil counts were significantly higher (t test, $p < 0.005$) in the Mn-exposed than in the control workers. These differences may be explained by an independent effect of Mn exposure and smoking.

Neither the mean values nor the cumulative frequency distributions of the level of whole blood serotonin and of the activity of monoamine oxidase in platelet-rich plasma (P-MAO) were significantly different between the control and Mn group. The mean ± SD (range) values for blood serotonin (expressed in three different ways) were for control and Mn-exposed workers, respectively: 178 ± 47 (27–287) vs 183 ± 54 (61–442) ng/ml, 73 ± 19 (12–130) vs 72 ± 21 (19–163) ng/10^8 platelets, and 100 ± 26 (16–179) vs 96 ± 30 (40–233) ng/μl of platelets. The P-MAO activity in the control and Mn group averaged 6.10 ± 1.91 (0.87–11.28) vs 5.90 ± 1.81 (0.64–12.15) nmol 4-HOQ/10^8 platelets × h, respectively. The prevalences of abnormal blood serotonin level and P-MAO activity were barely different between the control (5%) and Mn group (7%). Statistically significant increases of some serum parameters (ceruloplasmin, copper, ferritin, and calcium) were observed in the Mn group. The mean ± SD (range) values in control and Mn-exposed workers were respectively: for ceruloplasmin in serum (in mg/l) 471 ± 121 (198-840) vs 509 ± 104 (258–899) (t test, $p < 0.01$), for Cu in serum (in μg/100 ml) 101 ± 21 (52–161) vs 107 ± 18 (53–174) (t test, $p < 0.025$), for ferritin in serum (in ng/ml) 187 ± 177 (5–1298) vs 222 ± 187 (29–1298) and for calcium in serum (in mg/100 ml) 9.15 ± 0.47 (8.1–10.4) vs 9.57 ± 0.49 (8.7–11.1) (t test, $p < 0.001$). For serum calcium the prevalence of values exceeding 10 mg/100 ml was significantly higher (χ^2 test, $p < 0.02$) in the Mn group (16%) than in the control group (5%). For the renal parameters (β_2-microglobulin in serum, aminoaciduria, albuminuria, and RBP-U), no significant differences were found between the control and the manganese exposed workers.

Dose–Response Relationships

The significance of manganese in urine is still difficult to assess. On a group basis, however, Mn in blood seems more an index of body burden rather than of current exposure [Roels et al, 1987]. Therefore, the intensity of the various effects and the prevalences of abnormal values for the CNS and biological parameters were examined as a function of manganese concentration in blood and of duration of Mn exposure. On an individual basis, no statistically significant dose–effect relationship was found.

For the prevalence study, the control workers were considered as a separate group, while the workers exposed to manganese were classified into three subgroups according to Mn-B (< 1, 1–1.5, and > 1.5 μg/100 ml) or duration of exposure (< 3, 3–9, and > 9 years). The number of subjects in each subgroup comprises more or less one third of the Mn group.

The prevalences of abnormal values for simple reaction time, short-term memory, WBC, and neutrophil counts were not related to Mn-B. However, for eye–hand coordination, hand steadiness, and calcium in serum, the prevalences of elevated values increased steadily (χ^2 test; P at least < 0.02) with increasing concentration of

Mn in blood (Fig. 4). For the eye-hand coordination parameters, uncertainty and imprecision, there is some suggestion for the existence of a threshold level for Mn-B at about 1 μg/100 ml, while for hand steadiness and calcium in serum no threshold is evident. Only the prevalence of abnormal scores for one short-term memory parameter (standardized for age) exhibited a slight but statistically not significant tendency of increase with duration of exposure (see "sum of 5 trials in 30 sec" in Fig. 4).

We have also examined the prevalence of CNS symptoms and the prevalence of abnormal results for CNS and biological parameters as a function of the subjective estimation of integrated exposure to Mn (6 groups) by the chief foreman [Roels et al, 1987]. No statistically significant relationship was found (2×6 χ^2 test).

DISCUSSION

The present investigation compares the results of a cross-sectional epidemiological study among male workers exposed to Mn dust with those obtained in a matched control group. The duration of exposure to Mn was less than 20 years (1–19 years; average 7.1 years). In comparison to most of the earlier studies on workers from

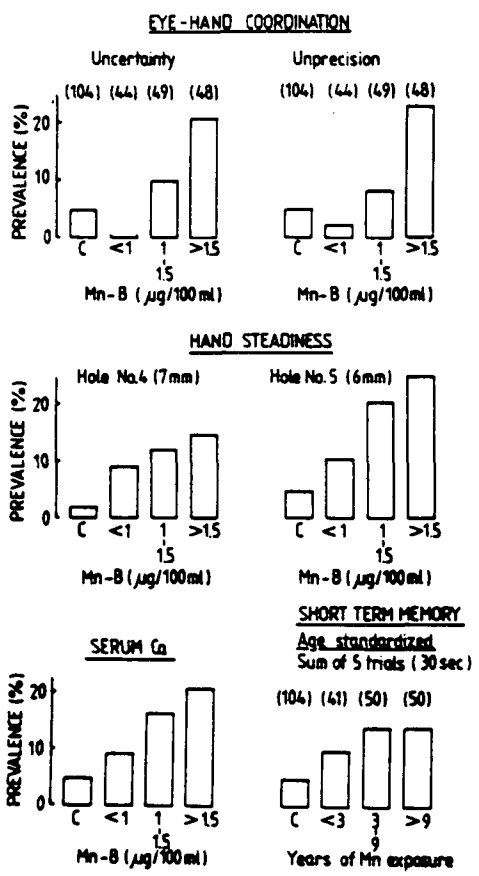

Fig. 4. Dose-response relationships between Mn-B and the prevalence of abnormal results for eye-hand coordination, hand steadiness and Ca in serum and dose-response relationship between duration of exposure to Mn and the prevalence of abnormal scores for audioverbal short-term memory. In parentheses the number of subjects in each group (C = control group and three subgroups of Mn-exposed workers).

mining and ore processing plants, dry-cell battery manufacture, or ferromanganese industry, the intensity of current exposure to airborne exposure Mn in the Mn oxide and salt producing plant studied can be considered moderate. Except for one outlying value (8.61 mg/m^3), all the levels of Mn-air were lower than 5 mg/m^3 (95th percentile 3.30 mg/m^3) and the average exposure intensity amounted to about 1 mg/m^3 (geometric mean 0.94; median 0.97 mg/m^3). However, in view of the increased manganese production over the last 15 years, it is possible that the average level of exposure to Mn dust was less than 1 mg/m^3 in the past.

Despite the efficiency of the homeostatic mechanisms [Britton and Cotzias, 1966; Leach, 1976; Abrams et al, 1976], the average Mn-B level in the Mn-exposed workers is more than twice as high as in the control workers, probably reflecting an increase in the manganese body burden [Roels et al, 1987].

Several adverse effects were detected in the Mn-exposed workers. A total number of about one hundred objective or subjective symptoms and functional or biological parameters were examined. Approximately 25% of them displayed less favorable results in the Mn-exposed workers in comparison to the control group. Depending on the parameter studied, the prevalence of abnormal results mostly ranged from 10 to 20% in the Mn group. These perturbations concerned the lung, the CNS, and a few biological parameters.

Several respiratory tract symptoms were more frequently reported in the Mn group than in the control group, especially cough in cold season and dyspnea during exercise. The prevalence of episodes of acute bronchitis was twice as high in the Mn group (about 40%) as in the control group. A similar questionnaire study was performed by Saric and Lucic-Palaic in 1977. As in our study, the prevalence of some respiratory symptoms such as phlegm and wheezing were not much different between Mn alloy workers (0.39–16.35 mg Mn/m^3) and the control workers (aluminum rolling mill, 0.05-0.07 μg Mn/m^3). The increasing tendency for the prevalence of respiratory symptoms with the extent of smoking habits, however, was most pronounced in the group of workers from the Mn alloy plant and the prevalence of chronic bronchitis was also highest in smokers of that plant. The authors suggested that Mn exposure and smoking might have a possible synergistic effect on the occurrence of respiratory symptoms. In the present study, no interaction effect between smoking and Mn exposure on several spirometric parameters was detected. By comparison with the control smokers, the Mn-exposed workers (smokers) exhibited a slight reduction in the mean values of FVC, FEV_1, and PEFR. These results, however, must be interpreted with caution because the control nonsmokers also exhibited a slight reduction of some ventilatory parameters possibly due to the fact that they were residing in an industrial area. In view of the matching of the control and Mn-exposed groups for socioeconomic and environmental factors, the present results suggest that Mn exposure has further decreased the pulmonary function of the exposed workers. Furthermore, it should be emphasized that the higher integrated smoking index in the control group might have partly masked the effect of Mn on the lung. The intensity of the Mn effect was rather mild, but we cannot exclude that a selection bias (departure of workers with pulmonary disease) existed. This could also explain that no dose–response relationship was found between duration of exposure and prevalence of respiratory signs and symptoms. Reported respiratory effects of Mn in humans cover a wide range of airborne Mn levels, eg Nogawa et al [1973] 0.003–0.011 mg/m^3 (schoolchildren), Saric and Lucic-Palaic [1977] 0.39–16.35 mg/m^3 (Mn

alloy workers), present study 0.07–8.61 mg/m^3. Although it is difficult to derive a no-effect level for manganese in air from the published results, the present study suggests that mild respiratory signs and symptoms may still occur at an average airborne Mn exposure of 1 mg/m^3.

The most characteristic signs of chronic Mn intoxication are those related to the CNS. The present investigation on active Mn workers was designed to detect early neurological effects of manganese. Except for a few nonspecific symptoms (fatigue, tinnitus, trembling of fingers, and increased irritability), the prevalences of the other complaints did not differ significantly between the exposed and control groups. Likewise, the standardized neurological examination was not very informative. Our observations are in agreement with those of Saric et al, [1977].

This epidemiological survey suggests that psychomotor tests constitute a sensitive tool for the early detection of adverse effects of Mn on the CNS. Significant changes were found for simple reaction time, audioverbal short-term memory capacity, and hand tremor. The average simple reaction time was longer and the effect of decreasing vigilance was much more pronounced in the Mn group. The Mn-exposed workers displayed a more important internal "central" variability since their standard deviation of simple reaction time was higher than that of the control group. Early manifestations of increased reaction time (foot-leg) have been reported by Siegl and Bergert (1982) in a group of 25 welders (CO_2 protection) exposed to relatively low airborne Mn levels (1.1–4 mg/m^3) for 16 years on the average. Statistically significant short-term memory deficits were also noticed in the Mn-exposed workers as well as significant preclinical alterations of hand tremor (eye-hand coordination, hand steadiness).

It has been suggested that Mn could interfere with the reproductive capacity. Diminished libido or impotence have been frequently reported in cases of chronic Mn exposure [Penalver, 1955; Chandra et al, 1974; Emara et al, 1971]. In the present study group, a statistically significant deficit in the number of children born to the Mn-exposed workers has been observed during the period of their exposure to Mn [Lauwerys et al, 1985]. In this context, it should be noticed that none of the control workers experienced impotence, whereas in the Mn group six workers reported this symptom.

The prevailing Mn exposure levels in the Mn plant studied did not cause alterations in the hematological parameters. The neutrophil count, however, was significantly higher in the Mn-exposed workers than in the control group. At levels of Mn in air higher than those observed in the present study, conflicting results were reported: Flinn et al [1941] observed leucopenia in 7 out of 11 Mn-intoxicated workers while Rodier [1966] mentioned a relative increase in lymphocytes and a decrease in polymorphonuclear leukocytes. Whitlock et al [1966] reported polymorphonuclear leukocytosis in two Mn steel workers with chronic neurological disease.

A few serum parameters were altered in the Mn-exposed workers. Our finding of a statistically significant increase of serum calcium in the Mn-exposed workers confirms previous observations [Jonderko et al, 1971; Chandra et al, 1974, 1981]. Experimental studies on Mn-intoxicated rats by Chandra et al [1973] demonstrated that the increase in serum calcium was accompanied by a decrease in the serum level of alkaline phosphatase activity and inorganic phosphate. The pathogenic mechanisms of these changes were not clear since no specific tissue abnormalities were found in parathyroids and bones. Since calcium is involved in the process of synaptic transmis-

sion, it is also possible that the interference of manganese with calcium metabolism plays some role in the neurotoxicity of manganese.

The present study also showed increased copper, ceruloplasmin, and ferritin levels in serum of the Mn-exposed workers. Ceruloplasmin may play a biological role in the transfer of iron from cells to plasma transferrin [Roeser et al, 1970] and may be involved in the oxidation of Mn^{2+} to Mn^{3+} prior to the binding to transferrin [Gibbons et al. 1976]. These observations suggest the existence of metabolic interactions between manganese, copper, and iron, but their mechanisms and their possible biological significance are still unknown. No statistically significant association was found between the various functional and biological changes. In other words, the Mn workers whose spirometric parameters were significantly decreased were not necessarily those who also exhibited reduction in psychomotor performances or increased serum Ca level.

Since the urinary excretion of Mn is more related to current Mn exposure and since it represents a very low fraction of the total amount excreted, it is not surprising that we did not find any dose–response relationship between Mn-U and the prevalence of abnormal results. Likewise, except for one age-standardized short-term memory parameter (sum of five trials in 30 sec), we could not demonstrate the existence of dose–response relationships between duration of exposure and the CNS parameters. Several reasons could explain this finding; either duration of employment is an inappropriate estimate of the total retained dose, or individual susceptibility is an important factor in Mn neurotoxicity, or a selection bias may have played a role (the most susceptible subjects may have left the factory—for the period 1964–1982, on the average 5–10% of the workforce left the factory each year for reasons other than retirement or dismissal by the employer). Siegl and Bergert (1982) reported a significant relationship between reaction time and duration of exposure in welders moderately exposed to Mn (1.1–4.0 mg/m^3). However, the duration of exposure of those welders was much longer (up to 30 years) than that of the workers in our study. With respect to blood manganese, there is some evidence of the existence of a few dose–response relationships and this seems to agree with the assumption that Mn-B reflects the body burden of Mn. The prevalence of two psychomotor disturbances (eye-hand coordination, hand steadiness) and that of increased calcium level in serum were found to be related to manganese concentration in blood. However, except for the eye-hand coordination parameters, there is no suggestion of the existence of a threshold for manganese in blood.

In summary, this study demonstrates that time-weighted average exposure to manganese dust (total dust) of about 1 mg/m^3 may still lead to the occurrence of preclinical adverse effects in the lungs and the central nervous system in some workers exposed for less than 20 years. The current average exposure level of 1 mg Mn/m^3 is most likely an overestimation for the exposure intensity in the past, since the production capacity of the plant gradually increased over the last 15 years. Therefore, the critical exposure level of airborne Mn (total dust) might be much lower than 1 mg/m^3. The results do not reveal a clear-cut biological limit value for manganese in blood, but since values exceeding those normally found in nonexposed population (95th percentile = 1 $\mu g/100$ ml) may still be associated with a slightly increased prevalence of abnormal findings, we are inclined to suggest a value of 1 $\mu g/100$ ml as a tentative guide for Mn-B.